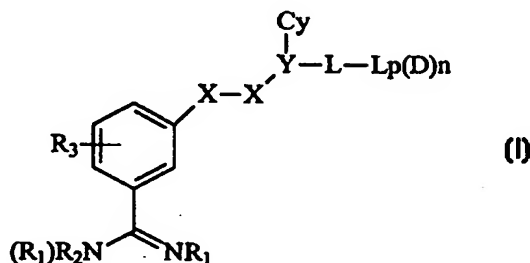




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/GB98/02605  <b>(22) International Filing Date:</b> 28 August 1998 (28.08.98)  <b>(30) Priority Data:</b> 9718392.5                      29 August 1997 (29.08.97)                      GB 9803173.5                      13 February 1998 (13.02.98)                      GB  <b>(71) Applicant (for all designated States except US):</b> PROTEUS MOLECULAR DESIGN LTD. [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> LIEBESCHUETZ, John, Walter [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). WYLIE, William, Alexander [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). WASZKOWYCZ, Bohdan [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). MURRAY, Christopher, William [GB/GB]; Beechfield House, Lyme Green Business		Park, Macclesfield, Cheshire SK11 0JL (GB). RIMMER, Andrew, David [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). WELSH, Pauline, Mary [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). JONES, Stuart, Donald [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). ROSCOE, Jonathan, Michael, Ernest [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). YOUNG, Stephen, Clinton [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB). MORGAN, Phillip, John [GB/GB]; Beechfield House, Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB).  <b>(74) Agents:</b> COCKBAIN, Julian et al.; Frank B. Dehn & Co., 179 Queen Victoria Street, London EC4V 4EL (GB).  <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  Published With international search report.
<b>(54) Title:</b> META-BENZAMIDINE DERIVATIVES AS SERIN PROTEASE INHIBITORS  <b>(57) Abstract</b>  The invention relates to serine protease inhibitor compound of formula (I), where R <sub>1</sub> and R <sub>2</sub> each independently is hydrogen or hydroxyl, alkoxy, alkyl, aminoalkyl, hydroxyalkyl, alkoxyalkyl, alkoxy carbonyl, acyloxymethoxycarbonyl or alkylamino optionally substituted by hydroxy, alkylamino, alkoxy, oxo, aryl or cycloalkyl; each R <sub>3</sub> independently is R <sub>1</sub> , R <sub>2</sub> , amino, halo, cyano, nitro, thiol, alkylthio, alkylsulfonyl, alkylsulphenyl, alkylsulphonamido, alkylaminosulphonyl, haloalkoxy and haloalkyl; each X independently is C, N, O or S atom or a CO, CR <sub>1</sub> , C(R <sub>1</sub> ) <sub>2</sub> or NR <sub>1</sub> group, at least one X being C, CO, CR <sub>1</sub> or C(R <sub>1</sub> ) <sub>2</sub> , with the proviso that if the benzamidine group is unsubstituted and the X-X group is -CH <sub>2</sub> C(R <sub>1</sub> ) <sub>2</sub> -, then R <sub>1</sub> is hydrogen or is attached to the alkylene carbon atom by a heteroatom; L is an organic linker group containing 1 to 5 backbone atoms selected from C, N, O and S, or a branched alkyl or cyclic group; Y is a nitrogen atom or a CR <sub>1</sub> group or Y and L taken together form a cyclic group; Cy is a saturated or unsaturated, mono or polycyclic, homo or heterocyclic group optionally substituted by groups R <sub>3</sub> or phenyl optionally substituted by R <sub>3</sub> ; Lp is a lipophilic organic group, selected from an alkyl, heterocyclic, alkenyl, alkaryl, cycloalkyl, polycycloalkyl, cycloalkenyl, aryl, aralkyl or haloalkyl group or a combination of two or more such groups optionally substituted by one or more of oxa, oxo, aza, thio, halo, amino, hydroxy or R <sub>3</sub> groups; D is a hydrogen bond donor group; and n is 0, 1 or 2 and salts thereof.		



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## META-BENZAMIDINE DERIVATIVES AS SERIN PROTEASE INHIBITORS

5 This invention relates to compounds which are inhibitors of serine proteases and to pharmaceutical compositions thereof and their use in the treatment of the human or animal body.

10 The serine proteases are a group of proteolytic enzymes which have a common catalytic mechanism characterized by a particularly reactive Ser residue. Examples of serine proteases include trypsin, tryptase, chymotrypsin, elastase, thrombin, plasmin, kallikrein, Complement C1, acrosomal protease, lysosomal protease, cocoonase,  $\alpha$ -lytic protease, protease A, protease B, 15 serine carboxypeptidase II, subtilisin, urokinase, Factor VIIa, Factor IXa, and Factor Xa. The serine proteases have been investigated extensively over a period of several decades and the therapeutic value of inhibitors of serine proteases is well understood.

20 Serine protease inhibitors play a central role in the regulation of a wide variety of physiological process including coagulation, fibrinolysis, fertilization, development, malignancy, neuromuscular patterning and inflammation. It is well known that 25 these compounds inhibit a variety of circulating proteases as well as proteases that are activated or released in tissue. It is also becoming clear that serine protease inhibitors inhibit critical cellular processes, such as adhesion, migration, free radical 30 production and apoptosis. In addition, animal experiments indicate that intravenously administered serine protease inhibitors, variants or cells expressing serine protease inhibitors, provide a protective effect against tissue damage.

35 Serine protease inhibitors have also been predicted to have potential beneficial uses in the treatment of disease in a wide variety of clinical areas such as

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oncology, neurology, haematology, pulmonary medicine, immunology, inflammation and infectious disease.

In particular serine protease inhibitors may be beneficial in the treatment of thrombotic diseases, asthma, emphysema, cirrhosis, arthritis, carcinoma, melanoma, restenosis, atheroma, trauma, shock and reperfusion injury.

Thus for example an inhibitor of Factor Xa has value as a therapeutic agent as an anticoagulant, e.g. in the treatment and prevention of thrombotic disorders. The use of a Factor Xa inhibitor as an anticoagulant is desirable in view of the selectivity of its effect. Many clinically approved anticoagulants have been associated with adverse events owing to the non-specific nature of their effects on the coagulation cascade.

Also, there are well-known associations of  $\alpha$ 1 protease inhibitor deficiency with emphysema and cirrhosis and C1 esterase inhibitor deficiency with angioedema.

We have now found that certain novel amidine compounds are particularly effective as inhibitors of serine proteases, especially proteases with negatively charged P1 specificity pockets, and most especially the serine proteases thrombin, trypsin, urokinase and Factor Xa.

Thus viewed from one aspect we provide serine protease inhibitor compounds having an m-benzamidine group and a lipophilic group coupled to a cyclic group-attached carbon or nitrogen atom (hereinafter the alpha atom), the coupling of the m-benzamidine group to the alpha atom being by a linker group providing a two backbone atom linking chain, the backbone atoms being selected from C, N, O and S and at least one being C, optionally wherein one or both of the backbone atoms form part of a cyclic group and the coupling of the lipophilic group to the alpha atom being by a linker group capable of separating the alpha atom from the



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lipophilic group by a range of 2.3 to 6.5 Å in length.

In the compounds of the invention, the lipophilic group is itself optionally substituted by a hydrogen bond donor group. Where this is the case, the lipophilic group and its linker preferably are conformable to separate by from 7.5 to 15.0 Å the alpha atom and the hydrogen bond donor atom of the donor group.

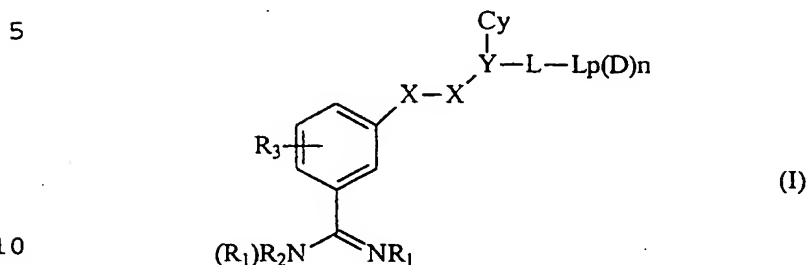
Where distances from the alpha atom to the lipophilic group or to the hydrogen bond donor atom are mentioned, these relate to the distances between the centre of the alpha atom and the centre of the first atom of the lipophilic group or the centre of the hydrogen bond donor atom.

Such distances can be calculated from crystallographic data for any given compound from the bond lengths and bond angles for individual bonds along the length of the molecule between the alpha atom and the first atom of the lipophilic group or the hydrogen bond donor atom. Similarly such distances may be calculated with reasonable accuracy from the bond lengths and bond angles known to be typical of such individual bonds.

The linker between the alpha atom and the lipophilic group may itself be a lipophilic moiety, e.g. an alkylene chain. The nature of the linker may vary considerably - the primary requirement is that it be conformable to place part or all of the lipophilic group at a desired distance away from the alpha atom. The lipophilic group thus desirably is able to occupy at least part of a space 2.3 to 15.0 Å from the alpha atom. The length of the linker generally corresponds to 1 to 5 backbone atoms and may be a chain, branched chain or cyclic linker (e.g. a cyclic amide or an aromatic heterocycle). In one embodiment the alpha atom forms part of a cyclic group which also forms the linker to the lipophilic group.

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Thus viewed from an alternative aspect the invention provides serine protease inhibitor compounds of formula I



(where  $R_1$  and  $R_2$  each independently is hydrogen or hydroxyl, alkoxy, alkyl, aminoalkyl, hydroxyalkyl alkoxyalkyl, alkoxy carbonyl, acyloxymethoxycarbonyl or alkylamino optionally substituted by hydroxy, alkylamino, alkoxy, oxo, aryl or cycloalkyl;

15

each  $R_3$  independently is  $R_1$ ,  $R_2$ , amino, halo, cyano, nitro, thiol, alkylthio, alkylsulphonyl, alkylsulphenyl, alkylsulphonamido, alkylaminosulphonyl, aminosulphonyl, haloalkoxy and haloalkyl;

20

each X independently is a C, N, O or S atom or a CO,  $CR_1$ ,  $C(R_1)_2$  or  $NR_1$  group, at least one X being C, CO,  $CR_1$  or  $C(R_1)_2$ , with the proviso that if the benzamidine group is unsubstituted (i.e. no non-hydrogen  $R_3$  group is present) and the X-X group is  $-CH_2C(R_1)_2-$  then  $R_1$  is hydrogen or is attached to the alkylene carbon atom by a heteroatom;

25

L is an organic linker group containing 1 to 5 backbone atoms selected from C, N, O and S, or a branched alkyl or cyclic group;

30

Y (the  $\alpha$ -atom) is a nitrogen atom or a  $CR_1$  group or Y and L taken together form a cyclic group;

Cy is a saturated or unsaturated, mono or poly cyclic, homo or heterocyclic group, preferably containing 5 to 10 ring atoms and optionally substituted by groups  $R_3$  or phenyl optionally substituted by  $R_3$ ;

35

Lp is a lipophilic organic group, e.g. an alkyl,

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heterocyclic, alkenyl, alkaryl, cycloalkyl, polycycloalkyl, cycloalkenyl, aryl, aralkyl or haloalkyl group or a combination of two or more such groups optionally substituted by one or more of oxa, oxo, aza, thio, halo, amino, hydroxy or  $R_3$  groups, preferably a group containing up to 25 carbon atoms;

D is a hydrogen bond donor group; and n is 0, 1 or 2);

or a physiologically tolerable salt thereof, e.g. a halide, phosphate or sulphate salt or a salt with ammonium or an organic amine such as ethylamine or meglumine.

In the compounds of the invention, where the alpha atom is carbon it preferably has the conformation that would result from construction from a D- $\alpha$ -aminoacid  $NH_2-CR_1(Cy)-COOH$  and a m-carboxyl benzamidine. Likewise the fourth substituent  $R_1$  at an alpha carbon is preferably a methyl or hydroxymethyl group or hydrogen.

In compounds of formula (I) it is envisaged that the unsubstituted or substituted amidino group could be replaced by a substituted or unsubstituted aminomethyl group although an amidino derivative is preferred.

In the compounds of the invention, unless otherwise indicated, aryl groups preferably contain 5 to 10 ring atoms optionally including 1, 2 or 3 heteroatoms selected from O, N and S; alkyl, alkenyl or alkynyl groups or alkylene moieties preferably contain up to 6 carbons; cyclic groups preferably have ring sizes of 3 to 8 atoms; and fused multicyclic groups preferably contain 8 to 16 ring atoms.

The linker group from the benzamidine group to the alpha atom is preferably selected from  $-CH=CH-$ ,  $-CONH-$ ,  $-CONR_1-$ ,  $-NH-CO-$ ,  $-NH-CH_2-$ ,  $-CH_2-NH-$ ,  $-CH_2O-$ ,  $-OCH_2-$ ,  $-COO-$ ,  $-OC=O-$  and  $-CH_2CH_2-$ . Preferably, the X moiety nearest to the alpha atom is an NH or O atom, most preferably a NH group. The X moiety alpha to the phenyl ring is preferably a carbon based group such as  $CH_3$  or

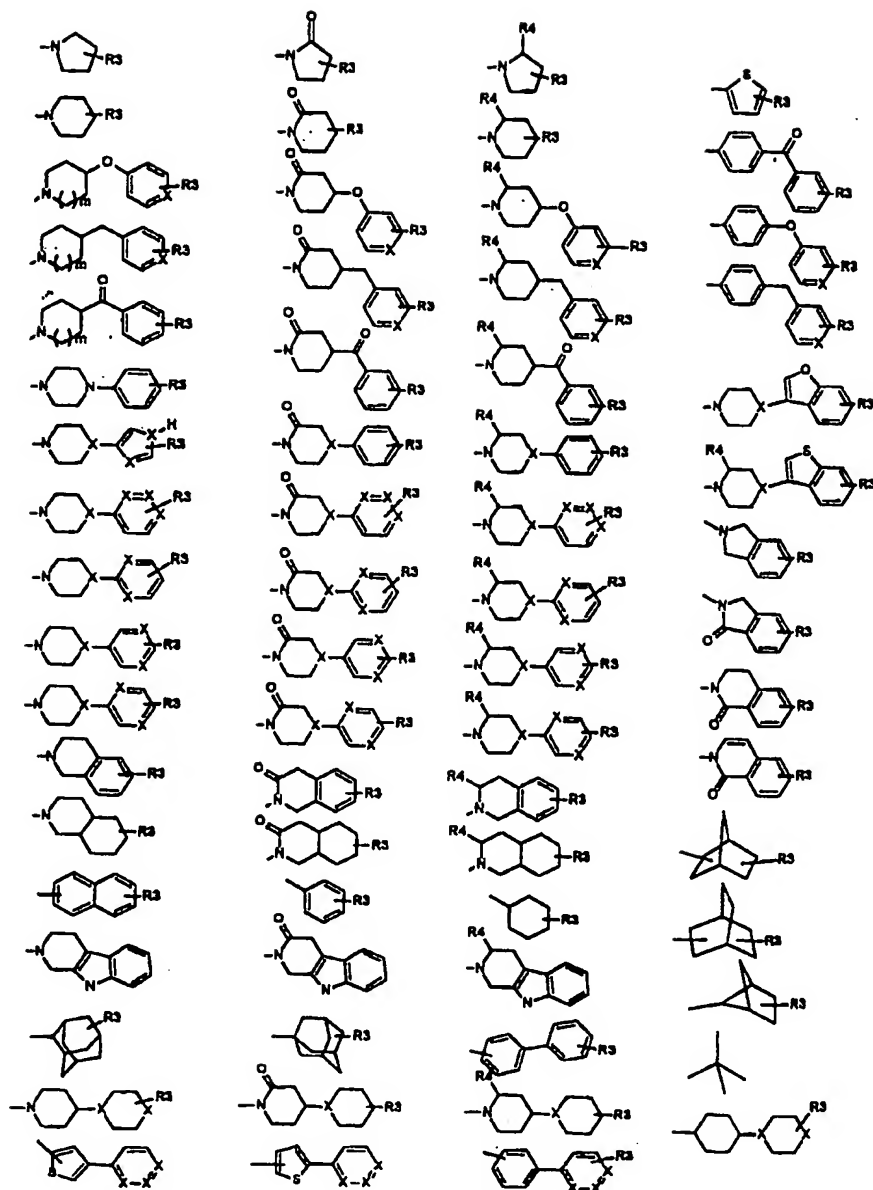
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CO, preferably CO. Thus a particularly preferred linker X-X is -CONH-.

The linker group from the alpha atom to the lipophilic group is preferably CO, CH<sub>2</sub>NH, CONR<sub>1</sub>(CH<sub>2</sub>)<sub>m</sub>,  
5 (CH<sub>2</sub>)<sub>m</sub>N(R<sub>1</sub>)CO(CH<sub>2</sub>)<sub>m</sub>, (CH<sub>2</sub>)<sub>m+2</sub>, CO(CH<sub>2</sub>)<sub>m</sub>, (CH<sub>2</sub>)<sub>m</sub>CO, (CH<sub>2</sub>)<sub>m</sub>OC=O, (CH<sub>2</sub>)<sub>m</sub>O or CH=CH(CH<sub>2</sub>)<sub>m</sub> (where each m is independently 0 or 1). The linker may be optionally branched, for example, to incorporate a polar functionality. In a preferred embodiment Y and L taken together form a cyclic group  
10 and the alpha atom is therefore a carbon atom. The cyclic group can be unsubstituted or substituted and can have a ring size of from 3 to 8 atoms. Preferably, the cyclic group is a cyclic amide, most preferably wherein the amide nitrogen of the cyclic amide group is bound to  
15 the lipophilic group.

The lipophilic group preferably comprises a cycloalkyl, azacycloalkyl, diazacycloalkyl, phenyl, naphthyl, adamantyl, decalinyl, tetrahydrodecalinyl, bicycloalkyl, mono- or diazabicycloalkyl, mono- or  
20 bicyclo heteroaromatic or a linear or branched alkyl, alkylene, alkenyl or alkenylene group all optionally substituted by one or more groups R<sub>3</sub>, or a combination of at least two such groups linked by a spiro linkage or a single or double bond or by C=O, O, S, SO, SO<sub>2</sub>, CONR<sub>1</sub>,  
25 NR<sub>1</sub>-CO-, NR<sub>1</sub> linkage. For example, representative lipophilic groups include a methyl-cyclohexyl, methylcyclohexylmethyl, methylphenylmethyl, phenylethyl, benzylpiperidinyl, benzoylpiperidinyl, bispiperidinyl or phenylpiperazinyl.

30 Most preferably, the lipophilic group is selected from



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wherein  $R_3$  is  $R_1$ , aryl or cycloalkyl;

$m$  represents 0 or 1;

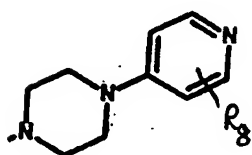
$R_4$  represents hydrogen,  $(CH_2)_wCOOH$ ,  $(CH_2)_wCONH_2$ ,  
 $(CH_2)_wCON\alpha\text{-AminoAcid}$ ;

5  $w$  represents an integer from 0 to 4; and

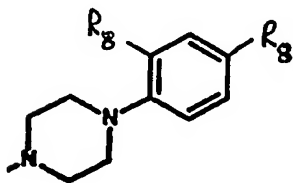
$X$  represents CH or N.

For example specific lipophilic groups include

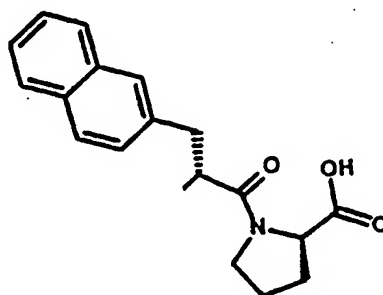
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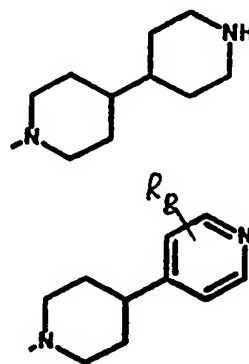
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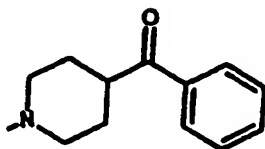
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35 especially when  $R_8$  represents H, OMe, F,  $NO_2$ , OH or Cl.

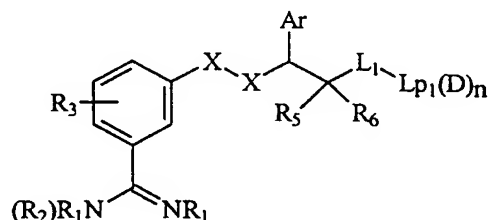
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The hydrogen bond donor group which may be attached to the lipophilic group preferably has a nitrogen or oxygen atom as the donor atom and conveniently is a hydroxyl group, a primary, secondary or tertiary amine, or a primary or secondary imine group (as part of an amidine or guanidine) or a saturated or unsaturated heterocyclic group containing a ring nitrogen, preferably a group containing 5 to 7 ring atoms. Where the donor atom is a ring nitrogen, the remote portion of the heterocyclic ring may be part of the lipophilic group.

The cyclic group attached to the alpha carbon is preferably an optionally  $R_3$  substituted phenyl or naphthyl group.

The benzamidino group is preferably an unsubstituted m-benzamidino group, or a substituted m-benzamidino group bearing metabolically labile groups such as acyloxymethoxycarbonyl, alkoxycarbonyl or hydroxy.

Accordingly, preferred compounds of the invention are of formula



(wherein  $R_1$ ,  $R_2$  and  $R_3$  are as hereinbefore defined,  $R_1$  and  $R_2$  preferably being hydrogen or one representing a metabolically labile group such as alkoxycarbonyl or hydroxy,  $R_3$  preferably being hydrogen, OH or  $\text{NH}_2$  and when other than hydrogen preferably being para to the amidine group);

$R_5$  and  $R_6$  are hydrogen or taken together with the

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carbon atom to which they are attached represent a carbonyl group;

Ar is an unsubstituted or substituted aryl group, preferably phenyl;

5 X-X is -CONH-, -CH<sub>2</sub>CH<sub>2</sub>-, CH<sub>2</sub>O-, -COO-, -CH<sub>2</sub>NH-, -OCH<sub>2</sub>- or -NHCH<sub>2</sub>-;

L<sub>1</sub> is a valence bond or an organic linker group containing 1 to 4 backbone atoms selected from C, N and O;

10 Lp<sub>1</sub> is a cycloalkyl, azacycloalkyl, diazacycloalkyl, phenyl, naphthyl, adamantyl, decalinyl, tetrahydrodecalinyl, bicycloalkyl, mono- or diazabicycloalkyl, mono- or bicyclo heteroaromatic or a linear or branched alkyl, alkylene, alkenyl or  
15 alkenylene group all optionally substituted by a group R<sub>3</sub>, or a combination of at least two such groups linked by a spiro linkage or a single or double bond or by C=O, O, S, SO, SO<sub>2</sub>, CONR<sub>1</sub>, NR<sub>1</sub>-CO-, NR<sub>1</sub> linkage. For example, representative lipophilic groups include a methyl-  
20 cyclohexyl, methylcyclohexylmethyl, bispiperidinyl, methylphenylmethyl, phenylethyl, benzylpiperidinyl, benzoylpiperidinyl or phenylpiperazinyl and those as hereinbefore described;

D is a hydrogen bond donor group;  
25 and n is 0, 1 or 2).

In one embodiment, L<sub>1</sub> comprises the backbone of an alpha amino acid, the lipophilic group being the side chain of the amino acid. The carboxyl part of the alpha amino acid may be optionally coupled via an amide bond  
30 to an amino acid or to a primary or secondary cyclic or acyclic alkyl amine or diamine or via an ester bond to primary or secondary alcohols.

In a preferred embodiment, L<sub>1</sub> represents a valence bond and the lipophilic group is bound directly to the carbonyl alpha to the alpha atom via a nitrogen atom  
35 which forms part of the lipophilic group. Suitable lipophilic groups in this case therefore include

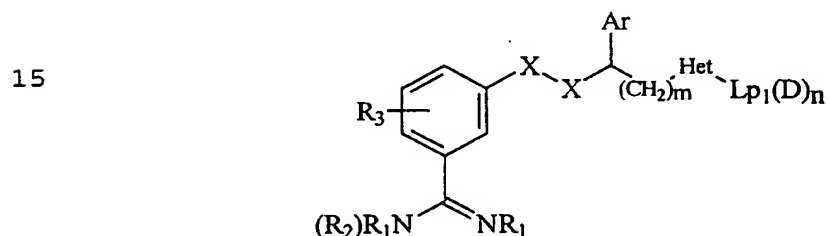


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piperidinyl, pyrrolidinyl and piperazinyl. In a preferred embodiment the piperidine or piperazinyl group is further substituted by a phenyl, benzyl, piperidine, pyridine or benzoyl group, optionally substituted on the phenyl ring by one or more  $R_3$  groups.

In a further embodiment, the lipophilic group has attached a group of the formula  $-\text{COOR}_1$  or  $-\text{CON-aminoacid}$  or ester derivative thereof.

In another embodiment the group binding the alpha carbon atom to the lipophilic group comprises a heterocyclic group. Accordingly, preferred compounds of the invention also include



20 (wherein  $R_1$ ,  $R_2$  and  $R_3$  are as hereinbefore defined  $R_1$  and  $R_2$  preferably being hydrogen or one representing a metabolically labile group such as alkoxycarbonyl or hydroxy,  $R_3$  preferably being hydrogen, OH or  $\text{NH}_2$  and when other than hydrogen preferably being para to the amidine group);

25 Ar is an unsubstituted or substituted aryl group, preferably phenyl;

X-X is  $-\text{CONH}-$ ,  $-\text{CH}_2\text{CH}_2-$ ,  $\text{CH}_2\text{O}-$ ,  $-\text{COO}-$ ,  $-\text{CH}_2\text{NH}-$ ,  $-\text{OCH}_2-$  or  $-\text{NHCH}_2-$ ;

30 m is 0, 1 or 2;

Het is a 5 or 6-membered heterocyclic group interrupted by 1, 2 or 3 heteroatoms selected from O, N and S optionally substituted by a group  $R_3$ ;

35  $\text{Lp}_1$  is a cycloalkyl, azacycloalkyl, diazacycloalkyl, phenyl, naphthyl, adamantyl, decalanyl, tetrahydrodecalanyl, bicycloalkyl, mono- or diazabicycloalkyl, mono- or bicyclo heteroaromatic or a

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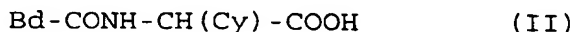
linear or branched alkyl, alkylene, alkenyl or alkenylene group all optionally substituted by a group  $R_3$ , or a combination of at least two such groups linked by a spiro linkage or a single or double bond or by C=O, O, S, SO, SO<sub>2</sub>, CONR<sub>1</sub>, NR<sub>1</sub>-CO-, NR<sub>1</sub> linkage. For example, representative lipophilic groups include a methyl-cyclohexyl, methylcyclohexylmethyl, methylphenylmethyl, phenylethyl, benzylpiperidinyl, benzoylpiperidinyl, bispiperidinyl or phenylpiperazinyl;

10 D is a hydrogen bond donor group;  
and n is 0, 1 or 2).

Where Het is a five membered ring, the two ring atoms at which it is connected are preferably separated by one ring atom. Where Het is a six-membered ring, the two ring atoms at which it is connected are preferably separated by one or two ring atoms. Representative heterocyclic groups include thiazole, oxazole, oxadiazole, triazole, thiadiazole or imidazole. Where the heterocyclic group is substituted by  $R_3$  this is preferably a COOH or COOR<sub>1</sub> connected to the heterocycle via a valence bond or alkylene chain.

20 In a further embodiment, the lipophilic group has attached a group of the formula -COOR<sub>1</sub> or -CON-aminoacid or ester derivative thereof.

25 The compounds of the invention may be prepared by conventional chemical synthetic routes, e.g. by amide bond formation to couple the benzamidine function to the alpha atom and to couple the lipophilic function to the alpha atom. Where the alpha atom is a carbon, the cyclic group-alpha atom combination may conveniently derive from an alpha amino acid with the benzamidine deriving from a m-amidino-benzoic acid. Amide formation from such reagents (in which the amidine function may if desired be protected during some or all of the synthesis steps) yields a compound of formula II.

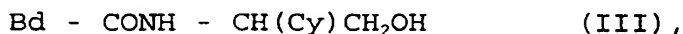


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(where Cy is as defined above and Bd is an optionally protected m-benzamidine group).

The lipophilic group (and optionally simultaneously the hydrogen bond donor) may then conveniently be introduced by reaction of a compound of formula II (or another analogous carboxylic acid) optionally after transformation into an activated form, e.g. an acid chloride or active ester, with a lipophilic group carrying an amine, hydroxylamine, hydrazine or hydroxyl group, e.g. to produce compounds with linkages of -CO-NR<sub>1</sub>-, -CO-NR<sub>1</sub>-O-, -CO-NR<sub>1</sub>-NR<sub>1</sub>- and -CO-O- from the alpha atom (where it is a carbon) to the lipophilic group. Where Y and L taken together form a cyclic amide group the lipophilic group can be conveniently introduced by reacting the compound of formula (II) with a lipophilic group carrying a secondary amine with an active side chain. Cyclisation can be base induced via nucleophilic attack of the alpha atom on a leaving group on the active side chain. If necessary the amide linkage can be reduced using an appropriate reducing agent employing the necessary protection depending on whether concurrent reduction of the carboxylic acid moiety is also desired. Alternatively a compound of formula II or another analogous carboxylic acid may be transformed into an alcohol by reaction with isobutylchloroformate and reduction with sodium borohydride.

Such an alcohol, e.g. of formula III



can be reacted to introduce the lipophilic group by reactions such as:

alkylation with an alkyl halide in the presence of a base;

reaction with diethyl azodicarboxylate/triphenylphosphine and a hydroxylated aryl compound;

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by reaction with an activated carboxylic acid (e.g. an acid chloride) or with a carboxylic acid and diethylazodicarboxylate/triphenylphosphine;

by reaction with an isocyanate; and

5 by treatment with methanesulphonyl chloride or trifluoromethanesulphonic anhydride and reaction with an amine, or with a thiol optionally followed by oxidation, e.g. with potassium metaperiodate or hydrogen peroxide.

10 In this way compounds with linkages of  $-\text{CH}_2-\text{O}-$ ,  $-\text{CH}_2-\text{O}-\text{CO}-$ ,  $-\text{CH}_2-\text{O}-\text{CO}-\text{NR}_1-$ ,  $-\text{CH}_2-\text{NR}_1-$ ,  $-\text{CH}_2-\text{S}-$ ,  $-\text{CH}_2-\text{SO}-$  and  $-\text{CH}_2-\text{SO}_2-$  between the alpha carbon and the lipophilic group may be produced.

Alternatively the alcohol can be oxidized to form a corresponding aldehyde (e.g. by oxidation with manganese dioxide or DMSO/oxalyl chloride or DMSO/ $\text{SO}_3$  or Dess-Martin reagent) which may be reacted to introduce the lipophilic group by reactions such as:

reaction with Wittig reagents or Horner-Emmons reagents, optionally followed by reduction of the resulting carbon:carbon double bond using  $\text{H}_2/\text{Pd}$ -carbon;

20 reaction with an organometallic, eg a Grignard reagent, optionally followed by reaction on the resulting hydroxyl group, such as oxidation (eg with  $\text{MnO}_2$ , DMSO/oxalyl chloride or Dess-Martin reagent),  
25 alkylation (eg with an alkyl halide in the presence of a base in a solvent such as DMF), arylation (eg with diethylazo dicarboxylate/triphenyl phosphine and a hydroxyaryl compound), ester formation (eg with an acid chloride or with a carboxylic acid and diethylazido dicarboxylate/triphenyl phosphine), or carbamate  
30 formation (eg with an isocyanate);

by reaction with an amine followed by reduction, e.g. with sodium cyanoborohydride;

by reaction with a hydrazine; or

35 by reaction with a carbazide.

In this way compounds with linkages of  $-\text{CH}=\text{CR}_1-$ ,  $-\text{CH}_2-\text{CHR}_1-$ ,  $-\text{CHOH}-$ ,  $-\text{CHR}_1-\text{O}-$ ,  $-\text{CHR}_1-\text{O}-\text{CO}-$ ,  $-\text{CHR}_1-\text{O}-\text{CO}-\text{NR}_1-$ ,

- 15 -

-CO-, -CH<sub>2</sub>-NR<sub>1</sub>-, -CH=N-NR<sub>1</sub>- and -CH=N-NR<sub>1</sub>-CO-NR<sub>1</sub>- between the alpha carbon and the lipophilic group may be produced.

5 The transformation of alcohol to amine referred to above may be used to produce an amine reagent for lipophilic group introduction, e.g. a compound Bd-CONH-CH(Cy)-CH<sub>2</sub>-NR<sub>1</sub>H.

10 Such an amine reagent may be reacted to introduce the lipophilic group, e.g. by acylation with an acid halide or activated ester, by reaction with isocyanate, by reaction with an isothiocyanate, or by reaction with a sulphonyl chloride. In this way compounds with linkages of -CH<sub>2</sub>NR<sub>1</sub>-CO-, -CH<sub>2</sub>-NR<sub>1</sub>-CO-NR<sub>1</sub>-, -CH<sub>2</sub>NR<sub>1</sub>-CS-NR<sub>1</sub>- and -CH<sub>2</sub>NR<sub>1</sub>-SO<sub>2</sub>- between the alpha carbon and the  
15 lipophilic groups may be produced.

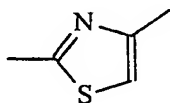
The transformation of acid to amide referred to above may be used to produce an amide reagent for introduction of the lipophilic group, e.g. a compound Bd-CONH-CH(Cy)-CON(R<sub>1</sub>)<sub>2</sub>.

20 Such amides may be reacted to introduce lipophilic groups, e.g. by reaction with a haloketone (e.g. phenacyl bromide). This provides a linkage



from alpha carbon to lipophilic group.

Analogously the amide may be transformed to a thioamide by reaction with Lawesson's reagent and then  
30 reacted with a haloketone to form a linkage

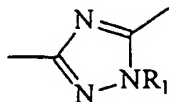


35 The amide reagent may likewise be transformed to a nitrile reagent by dehydration, e.g. with trifluoroacetic anhydride. The nitrile reagent may be

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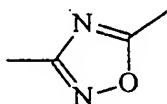
reacted with hydrazine then with acyl halide and then cyclized, (e.g. with trifluoroacetic anhydride) to produce a linkage

5



Alternatively it may be treated with hydroxylamine then reacted with acyl halide and cyclized (e.g. with trifluoroacetic anhydride) to produce a linkage

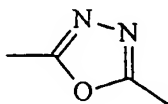
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The hydrazide produced by reaction of a carboxylic acid reagent with hydrazine discussed above may likewise be used as a reagent for lipophilic group introduction, e.g. as a compound of formula  $\text{Bd-CONH-CH(Cy)-CO-NR}_1\text{-N(R}_1)_2$ .

Thus the hydrazide reagent can be reacted with an acyl halide and cyclized, e.g. with trifluoroacetic anhydride to yield a linkage

20



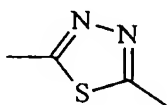
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or reacted with an acyl halide or an isocyanate to yield linkages  $\text{-CO-NR}_1\text{-NR}_1\text{-CO-}$  and  $\text{-CO-NR}_1\text{-NR}_1\text{-CO-NR}_1\text{-}$  respectively.

Alternatively the hydrazide may be transformed by reaction with Lawesson's reagent and then reacted with an acyl halide and cyclized (e.g. with trifluoroacetic anhydride) to produce the linkage

30

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An alternative route to these compounds is to carry out any of the above chemical reactions to incorporate the lipophilic group (an optional H bond donor) into a protected intermediate such as a compound of formula (IV).



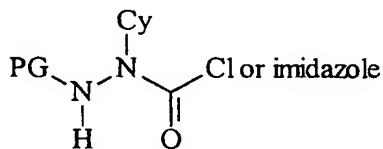
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PG = Protecting group

The protecting group may then be removed before coupling of the m-amidino benzoic acid (optionally protected).

A starting reagent for lipophilic group introduction where the alpha atom is nitrogen may be produced for example by reaction of a beta protected hydrazine (such protection to be chosen as to be compatible with the subsequent reagents to be employed) with phosgene, diphosgene, triphosgene or N,N'-carbonyl diimidazole to give a reactive compound of the type:

25



PG = Protecting group

30

This intermediate may be used as has been described above for the carboxylic starting reagents where the alpha atom is carbon.

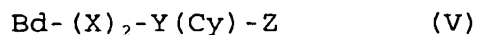
Removal of the protecting group by standard methods and coupling with an activated m-carboxyl-benzamidine will give compounds of the type

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(where Bd, X, Y, Cy, L, Lp and D are as defined above).

Thus viewed from a further aspect the invention provides a process for the preparation of a compound according to the invention which process comprises coupling a lipophilic group to a compound of formula (V)



(where Bd, X, Y and Cy are as defined above and Z is a reactive functional group), and optionally subsequently coupling a hydrogen bond donor group to said lipophilic group.

The compounds of the invention may be administered by any convenient route, e.g. into the gastrointestinal tract (e.g. rectally or orally), the nose, lungs, musculature or vasculature or transdermally. The compounds may be administered in any convenient administrative form, e.g. tablets, powders, capsules, solutions, dispersions, suspensions, syrups, sprays, suppositories, gels, emulsions, patches etc. Such compositions may contain components conventional in pharmaceutical preparations, e.g. diluents, carriers, pH modifiers, sweeteners, bulking agents, and further active agents. Preferably the compositions will be sterile and in a solution or suspension form suitable for injection or infusion. Such compositions form a further aspect of the invention. Viewed from this aspect the invention provides a pharmaceutical composition comprising a serine protease inhibitor according to the invention together with at least one pharmaceutically acceptable carrier or excipient.

Viewed from a further aspect the invention provides the use of a serine protease inhibitor according to the invention for the manufacture of a medicament for use in a method of treatment of the human or non-human animal body (e.g. a mammalian, avian or reptilian body) to combat (i.e. treat or prevent) a condition responsive to



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said inhibitor.

Viewed from a further aspect the invention provides a method of treatment of the human or non-human animal body (e.g. a mammalian, avian or reptilian body) to combat a condition responsive to a serine protease inhibitor (e.g. a condition such as a thrombotic disorder responsive to a factor Xa inhibitor), said method comprising administering to said body an effective amount of a serine protease inhibitor according to the invention.

The dosage of the inhibitor compound of the invention will depend upon the nature and severity of the condition being treated, the administration route and the size and species of the patient. However in general, quantities of from 0.01 to 100  $\mu\text{mol/kg}$  bodyweight will be administered.

All publications referred to herein are hereby incorporated by reference.

The invention will now be described further with reference to the following non-limiting Examples.

### Experimental

Abbreviations used follow IUPAC-IUB nomenclature. Additional abbreviations are Hplc, high-performance liquid chromatography; DMF, dimethylformamide; DCM, dichloromethane; HOAt, 1-hydroxy-7-azabenzotriazole; HATU, [O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate]; Fmoc, 9-Fluorenylmethoxycarbonyl; HOBt, 1-hydroxybenzotriazole; TBTU, 2-(1H-(benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumtetrafluoroborate; DIPEA, diisopropylethylamine; Boc, tertiary butyloxycarbonyl; DIPCI, diisopropylcarbodiimide; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; TEA, triethylamine; Rink linker, p-[(R,S)- $\alpha$ -[1-(9H-Fluoren-9-yl)methoxyformamido]-2,4-dimethoxybenzyl]phenyl acetic acid; TFA,

- 20 -

trifluoroacetic acid; MALDI-TOF, Matrix assisted laser desorption ionisation - time of flight mass spectrometry; and RT, retention time. Unless otherwise indicated, amino acid derivatives, resins and coupling reagents were obtained from Novabiochem (Nottingham, UK) and other solvents and reagents from Rathburn (Walkerburn, UK) or Aldrich (Gillingham, UK) and were used without further purification.

10 **Purification:** Purification was by gradient reverse phase Hplc on a Waters Deltaprep 4000 at a flow rate of 50 ml/min. using a Deltapak C18 radial compression column (40 mm x 210 mm, 10-15 mm particle size). Eluant A consisted of aqTFA (0.1%) and eluant B 90% MeCN in  
15 aqTFA(0.1%) with gradient elution (Gradient 1, 0 min. 20%B then 20% to 100% over 36 min., Gradient 2, 0 min. 5%B for 1 min. then 5%B to 20%B over 4 min., then 20% to 60% over 32 min. or Gradient 3, 0 min. 20%B then 20% to 100% over 15 min.). Fractions were analysed by  
20 analytical Hplc and MALDI-TOF before pooling those with >95% purity for lyophilisation.

**Analysis:** Analytical Hplc was on a Shimadzu LC6 gradient system equipped with an autosampler, a variable  
25 wavelength detector at flow rates of 0.4 ml/min. Eluents A and B as for preparative Hplc. Columns used were Techogell15 C18 (2x150mm) (Hplc Technology), Jupiter 5 C18 column (2.1x150 mm, 5µm particle size) (Phenomenex) and Kromasil C4 (2.0x150 mm, 5 mm) (HPLC  
30 Technology). Purified products were further analysed by MALDI-TOF and nmr.

All Fmoc-protected amino acids were purchased where available or prepared by known literature methods (1)  
35 with the exception of the following novel amino acid:

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**Preparation of (D,L)-N-Fluorenylmethoxycarbonyl-4-phenylphenylglycine.**

A solution of 4-biphenylcarboxaldehyde (4.6g 25mmol),  
5 sodium cyanide (3.68g 75mmol) and ammonium carbonate  
(9.60g 100mmol) in 50% aqueous ethanol (175ml) was  
heated at 50°C for 20 hours. The reaction mixture was  
cooled, concentrated under reduced pressure and  
acidified to pH 2.0 with concentrated hydrochloric acid  
10 (fume hood). The intermediate 4-(4-phenylphenyl)-2,5-  
imidazolidione was collected by filtration, washed  
with dilute (0.5%) HCl and dried before using as crude  
in the next stage. (Filtrates were retained and treated  
with sodium hypochlorite solution before disposal)  
15 The intermediate 4-(4-phenylphenyl)-2,5-imidazolidione  
was refluxed in 16% aqueous sodium hydroxide (100ml, 16%  
w/v) for 24h. The reaction mixture was then filtered,  
cooled, diluted with water (100ml) and then shaken with  
ethyl acetate and separated. The aqueous solution was  
20 adjusted to pH 5.1 with concentrated hydrochloric acid  
and the solid obtained collected by filtration, washed  
with a little water and dried to give 4-  
phenylphenylglycine (2.36g, 42%). <sup>1</sup>H nmr (d<sub>6</sub> DMSO)  
consistent with desired product.  
25 To a vigorously stirred solution of 4-  
phenylphenylglycine (500mg 2.2mmol) in DCM (20ml) was  
added DIPEA (614μl 4.4mmol) and then, carefully,  
chlorotrimethylsilane (558μl 4.4mmol) and the mixture  
refluxed for 1.5h. The reaction mixture was cooled in  
30 an ice bath and fluorenylmethoxycarbonyl chloride (742mg  
2.2mmol) was added in one portion. After stirring at 0°C  
for 20min the ice bath was removed and stirring was  
continued for 1.5h. The reaction mixture was  
concentrated under reduced pressure and the residue  
35 stirred with a mixture of diethyl ether (20ml) and  
saturated sodium carbonate solution (30ml). A yellow  
solid which failed to dissolve in either layer was taken

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up in water (20ml) and acidified to pH 1 with dilute hydrochloric acid. The mixture was then extracted with ethyl acetate and the organic layer washed with water (2 x 20ml), dried with magnesium sulphate and evaporated to dryness. Recrystallisation from ethanol/water afforded (D,L)-N-fluorenylmethyloxycarbonyl-4-phenylphenylglycine (640mg, 65%).

<sup>1</sup>H nmr (d<sub>6</sub> DMSO) 8.25 (1H, d, aromatics); 7.89 (2H, d, aromatics); 7.76 (2H d, aromatics); 7.67 (3H, d, aromatics); 7.57-7.25 (6H, m, aromatics); 5.23 (1H, d, -NH); ~4.27 (3H, m, H<sub>a</sub> + CH<sub>2</sub>).

#### Preparation of 3-Amidinobenzoic acid TFA salt

3 Cyanobenzoic acid (10g, 68 mmol) was refluxed in ethanol (300ml) on an isomantle fitted with reflux condenser and soxhlet extractor, the thimble being filled with A4 molecular sieve. Reflux was continued for 10 hours. The heating was then removed and the solution allowed to cool. The solution was then cooled in an ice bath and saturated with hydrogen chloride gas. The sealed flask was allowed to stand overnight then evaporated to dryness. To the dry product was added saturated ammonia/ethanol solution (400ml) and the flask sealed and allowed to stand overnight. The solution was then evaporated to dryness and then treated with 2M sodium hydroxide solution (3 eq., 102ml), the resulting solution was stirred for 2 hours then extracted with ethylacetate (100ml). The aqueous layer was then acidified, with 10% aq hydrochloric acid (200ml) in one lot and extracted with ethylacetate (100ml). Concentrated ammonia solution was added to pH 14 and the solution cooled in the fridge overnight. The precipitate formed was filtered off and washed with water, dissolved in 10%TFA water and lyophilised to a white powder (12g).

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<sup>1</sup>H nmr (D<sub>2</sub>O) 8.40 (1H, s); 8.30 (1H, d, J=7.5Hz); 8.00 (1H, d, J=7.5Hz); 7.72 (1H, t).

### Synthesis of inhibitors

5

**Method 1:** Using a solid phase strategy on a Protein Technologies, Symphony Multiple Peptide Synthesiser by attachment of bis amino compounds to Peg-2-chlorotrityl chloride resin: 2-Chlorotrityl chloride resin was typically treated with greater than 2 fold excess of the di-amine in dry DCM. The resin was further modified by the attachment of acids. Activation of Fmoc protected amino acid (2-5eq) was by TBTU/ DIPEA, all couplings (minimum 120 min.) were carried out in DMF. Deprotection of the Fmoc group was achieved with 20% (v/v) piperidine in DMF. Other acid substituents were added as the HOBt or HOAt esters either by activation with HBTU/HATU or DIPCI with or without Boc protection of amino groups. Cleavage of the products from the resin was by treatment (30 min., ambient) with 10% (v/v) triethylsilane in TFA, filtration, evaporation and trituration with diethylether.

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Synthesis using the Symphony Multiple Peptide Synthesiser.

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The Symphony Multiple Peptide Synthesiser is charged with DMF, DCM, TBTU in DMF (450 mM), DIPEA in DMF (900 mM), 20% (v/v) piperidine in DMF. Resins are held in plastic reaction vessels that allow the introduction of reagents and solvents and nitrogen for agitation or air drying.

35

A typical synthesis cycle on the Symphony is as follows:-

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The reaction vessel containing the resin (0.1 mmol) is charged with the Fmoc protected amino acid (0.5 mmol) and then this is dissolved in DMF (2.5ml), treated with TBTU (0.56 mmol, 1.25ml) and DIPEA (1.1 mmol, 1.25ml) and agitated with nitrogen for 2 hours (agitation times may vary). After coupling the resin is washed with DMF (6x 5ml) then deprotected with 20% (v/v) piperidine in DMF (2x 5ml for 1 min.each, then 1x 5ml for 8 min.) the resin is then washed with DMF (6x 5ml).

10

**Example 1:****3-Amidino-benzoyl-D-phenylglycine 4-aminomethylcyclohexyl methylamide**

Bis-1,4 aminomethylcyclohexane(2ml) was added to 2 chlorotrityl chloride resin (1.2 mmol/g, 0.73g) pre swollen in dry DCM (4ml). After 2h the resin was washed with DCM (6x5ml), DMF (6x5ml) and DCM (6x5ml). The resin was then air dried to allow aliquots to be taken.

20 The bis-1,4-aminomethylcyclohexane 2-chlorotrityl chloride resin (0.1 mmol) was treated with Fmoc-D-Phenylglycine (0.5 mmol, 187mg), DMF(2.5ml); TBTU in DMF(1.25ml of a 450mM solution) and DIPEA in DMF (1.25ml of a 900 mM solution). The mixture was agitated with 25 nitrogen for 2 hours. Deprotection and washing as above.

HOBt (0.5 mmol, 68 mg) dissolved in DMF(4ml) at 0°C was treated with DIPCI (0.5 mmol, 80µl), for 10 min. 3-amidinobenzoic acid TFA salt (0.5 mmol, 139 mg.) was 30 added and stirring continued for 10 min. at room temperature. The mixture was transferred to the reaction vessel on the Symphony and agitated for 10 hours with nitrogen. The resin was washed with DMF (6x5ml), DCM (6x5ml) and air dried. The product was cleaved from the 35 resin with 10% triethylsilane in TFA (10ml) for 30 minutes, the resin filtered off and the TFA solution evaporated to dryness and triturated with diethyl ether

- 25 -

to give the crude product. The crude product was dissolved in water (10ml), filtered and purified by preparative reverse phase Hplc.

5 <sup>1</sup>H nmr (D<sub>2</sub>O) Mixture of cyclohexyl cis and trans isomers  
8.09 (1H, s); 8.05 (1H, d, J=7.5Hz); 7.90 (1H, d,  
J=7.5Hz); 7.66 (1H, t, J=7.5Hz); 7.43 (5H, m); 5.47 (1H,  
s); 3.05 (2H, m); 2.78 (2H, m); 1.48 (7H, m); 0.86 (3H,  
m) MS TOF 422 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1,  
10 water/acetonitrile/TFA) rt 13.45 min. (major) and 13.62  
min. (minor).

Compounds made by the above method:-

15 **Example 2.**

**3-Amidino-benzoyl-DL-3-chloro-phenylglycine-4-aminomethylcyclohexyl methylamide**

<sup>1</sup>H nmr (D<sub>2</sub>O) Mixture of cyclohexyl cis and trans isomers  
8.19 (1H, s); 8.15 (1H, d, J=7.5Hz); 8.04 (1H, d,  
20 J=7.5Hz); 7.90 (1H, t, J=7.5Hz); 7.53 (4H, m); 5.63 (1H,  
s); 3.18 (2H, m); 2.90 (2H, m); 1.66 (7H, m); 1.00 (3H,  
m). (MS TOF 457 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2,  
water/acetonitrile/TFA) rt 16.53 min.

25 **Example 3.**

**3-Amidino-benzoyl-DL-4-methoxyphenylglycine-4-aminomethylcyclohexyl methylamide**

<sup>1</sup>H nmr (D<sub>2</sub>O) Mixture of cyclohexyl cis and trans isomers  
8.19 (1H, s); 8.12 (1H, d, J=7.5Hz); 8.01 (1H, d, J-  
30 7.5Hz); 7.80 (1H, t, J=7.5Hz); 7.50 (2H, d); 7.10,  
(2H, d); 5.53 (1H, s); 3.86 (3H, s); 3.13 (2H, m); 2.90  
(2H, m); 1.66 (7H, m); 0.95 (3H, m). (MS TOF 452 (M+1<sup>+</sup>).  
Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA)  
rt 13.87 min. (major) and 14.10 min. (minor).

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**Example 4.**

**3-Amidino-benzoyl-DL-4-hydroxyphenylglycine 4-aminomethylcyclohexyl methylamide**

<sup>1</sup>H nmr (D<sub>2</sub>O) Mixture of cyclohexyl cis and trans isomers  
5 7.40 (1H, d); 7.25 (2H, dd); 7.08 (1H, d); 7.02 (1H, d); 6.88 (2H, m); 6.82 (1H, d); 5.28 (1H, d); 5.10 (1H, d); 3.03 (2H, m); 2.83 (2H, m); 1.50 (7H, m); 0.85 (3H, m). MS TOF 438 (M+1<sup>+</sup>) Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt 9.69 min. (major) and 14.10  
10 min. (minor).

**Example 5.**

**3-Amidino-benzoyl-DL-1-naphthylglycine 4-aminomethylcyclohexyl methylamide**

<sup>1</sup>H nmr (D<sub>2</sub>O) Mixture of cyclohexyl cis and trans isomers  
15 8.40 (1H, s); 8.25 (4H, t); 8.132 (1H, d, J=7.5Hz); 7.81 (4H, m); 6.53 (1H, s); 3.70-3.10 (2H, m); 2.99 (2H, m); 2.0-1.3 (7H, m); 1.07 (3H, m). (MS TOF 472 (M+1<sup>+</sup>)). Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt 17.62 min. (major) and 18.03  
20 min. (minor).

**Example 6.**

**3-Amidino-benzoyl-DL-4-phenyl phenylglycine 4-aminomethylcyclohexyl methylamide**

<sup>1</sup>H nmr (D<sub>2</sub>O) Mixture of cyclohexyl cis and trans isomers  
25 8.11 (2H, m); 7.94 (1H, d, J=7.5Hz); 7.71 (5H, m); 7.51 (5H, m); 5.58 (1H, s); 3.3-2.90 (2H, m); 2.90-2.65 (2H, m); 1.8-1.2 (7H, m); 0.85 (3H, m). MS TOF 498 (M+1<sup>+</sup>)  
30 Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/ TFA) rt 19.37 min. (major), 21.69 min. (minor).

**Example 7.**

**3-Amidino-benzoyl-DL-4-trifluoromethyl phenylglycine 4-aminomethylcyclohexyl methylamide**

<sup>1</sup>H nmr (D<sub>2</sub>O) Mixture of cyclohexyl cis and trans isomers  
35 8.12 (1H, s); 8.07 (1H, d, J=7.5Hz); 7.93 (1H, d,



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J=7.5Hz); 7.8-7.6 (5H, m); 5.61 (1H, s); 3.30-2.70 (4H, m); 1.50 (7H, m); 0.85 (3H, m). MS TOF 490 (M+1<sup>+</sup>) Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt 19.37 min.

5

**Example 8.****3-Amidino-benzoyl-R,S-3-phenyl  $\beta$ -alanine 4-aminomethylcyclohexyl methylamide**

<sup>1</sup>H nmr D<sub>2</sub>O Mixture of cyclohexyl cis and trans isomers.

10 8.08 (2H, m); 7.96 (1H, d, J=7.5Hz); 7.73 (1H, t, J=7.5Hz); 7.44 (5H, m); 5.43 (1H, t, J=7.9Hz); 3.50 (2H, m); 2.88 (4H, m); 1.6-1.0 (7H, m); 0.77 (2H, m). MS TOF 436 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt 13.22 min. (major), 13.61  
15 (minor)

**Example 9.****3-Amidino-benzoyl-DL-3-indolylglycine 4-aminomethylcyclohexyl methylamide**

20 <sup>1</sup>H nmr (D<sub>2</sub>O) Mixture of cyclohexyl cis and trans isomers

8.1-7.85 (3H, m); 7.65 (1H, m); 7.47 (4H, m); 7.33 (1H, m); 4.85 (m, partially obscured by solvent); 4.3.3.80 (4H, m); 3.05 (2H, m); 2.78 (3H, m); 1.8-1.0 (7H, m); 1.0-0.65 (3H, m). MS TOF 451 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt 10.12 min.  
25

**Example 10.****3-Amidino-benzoyl-DL-piperonylglycine 4-aminomethylcyclohexyl methylamide**

30 <sup>1</sup>H nmr D<sub>2</sub>O Mixture of cyclohexyl cis and trans isomers.

8.13 (2H, m); 8.00 (1H, d, J=7.5Hz); 7.72 (1H, t, J=7.5Hz); 6.96 (5H, m); 5.98 (2H, s); 5.44 (1H, s); 3.4-2.7 (4H, m); 1.9-1.25 (7H, m); 1.1-0.8 (3H, m). MS TOF 466 (M+1<sup>+</sup>) Hplc (Kromasil C4, Gradient 2, water/acetonitrile/ TFA) rt 12.62 min.  
35

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**Example 11.****3-Amidino-benzoyl-DL-3-methyl phenylglycine 4-aminomethylcyclohexyl methylamide**<sup>1</sup>H nmr D<sub>2</sub>O Mixture of cyclohexyl cis and trans isomers.

5 8.09 (2H, m); 7.96 (1H, d, J=7.5Hz); 7.70 (1H, t, J=7.5Hz); 7.25 (4H, m); 5.50 (1H, s); 3.4-2.7 (4H, m); 2.31 (3H, s); 1.9-1.1 (7H, m); 1.1-0.7 (3H, m). MS TOF 436 (M+1<sup>+</sup>) Hplc (Kromasil C4, Gradient 2, water/acetonitrile/ TFA) rt 14.41 min.

10

**Example 12.****3-Amidino-benzoyl-DL-2-naphthylglycine 4-aminomethylcyclohexyl methylamide**<sup>1</sup>H nmr D<sub>2</sub>O Mixture of cyclohexyl cis and trans isomers.

15 7.98 (2H, m); 7.83 (4H, m); 7.56 (1H, t, J=7.5Hz); 7.45 (4H, m); 5.58 (1H, s); 3.25-2.55 (4H, m); 1.7-0.9 (7H, m); 0.9-0.5 (3H, m). MS TOF 472 (M+1<sup>+</sup>) Hplc (Kromasil C4, Gradient 2, water/acetonitrile/ TFA) rt 17.44 min. (major) 17.69 (minor).

20

**Example 13.****3-Amidino-benzoyl-1-aminocyclopentane-N- 4-aminomethylcyclohexyl methyl-1-carboxamide**<sup>1</sup>H nmr D<sub>2</sub>O Mixture of cyclohexyl cis and trans isomers.

25 7.92 (2H, m); 7.81 (1H, d, J=7.5Hz); 7.57 (1H, t, J=7.5Hz); 3.6-3.1 (2H, m); 2.91 (2H, d, J=6.8Hz); 2.68 (2H, d, J=6.8Hz); 2.2-1.8 (4H, m); 1.8-1.2 (13H, m); 1.0-0.65 (3H, m). MS TOF 400 (M+1<sup>+</sup>) Hplc (Kromasil C4, Gradient 2, water/acetonitrile/ TFA) rt 10.87 min.

30

**Example 14.****3-Amidino-benzoyl-DL-cyclohexylglycine 4-aminomethylcyclohexyl methylamide**<sup>1</sup>H nmr (D<sub>2</sub>O) Mixture of cyclohexyl cis and trans isomers

35 8.10 (1H, s); 8.05 (1H, d, J=7.5Hz); 7.85 (1H, d, J=7.5Hz); 7.66 (1H, t, J=7.5Hz); 4.25 (1H, d); 3.05 (2H, m); 2.78 (2H, m); 1.48 -0.86 (19H, m) MS TOF 428 (M+1<sup>+</sup>).

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Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA)  
rt 14.76 min. (major) and 15.09 min. (minor).

Example 15.

- 5     3-Amidino-benzoyl-L-phenylglycine 4-  
      aminomethylcyclohexyl methylamide  
      MS TOF 422 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2,  
      water/acetonitrile/ TFA) rt 10.06 min.

10    Example 16.

      3-Amidino-benzoyl-D-phenylglycine 3-  
      aminomethylcyclohexyl methylamide  
      MS TOF 422 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2,  
      water/acetonitrile/ TFA) rt 8.81 min.

15

      Example 17.

- 3-Amidino-benzoyl-L-phenylglycine 3-  
      aminomethylcyclohexyl methylamide  
      MS TOF 422 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2,  
20    water/acetonitrile/ TFA) rt 8.79 min.

      Example 18.

- 3-Amidino-benzoyl-DL-2-thienylglycine 4-  
      aminomethylcyclohexyl methylamide  
25    MS TOF 429 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2,  
      water/acetonitrile/TFA) rt 12.73 min.

      Example 19.

- 3-Amidino-benzoyl-DL-4-chloro-phenylglycine 4-  
30    aminomethylcyclohexyl methylamide  
      MS TOF 457 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2,  
      water/acetonitrile/TFA) rt 15.89 min.

      Example 20

- 35    3-Amidino-benzoyl-D-phenylglycine 4-  
      aminomethylbenzylamide  
      MS TOF 416 (M+1<sup>+</sup>) Hplc (Techogel15 C18, Gradient 2,

- 30 -

water/acetonitrile/ TFA) rt 12.34 min.

**Example 21.**

5     **3-Amidino-benzoyl-D-phenylglycine 3-aminomethylbenzylamide**

MS TOF 416 (M+1<sup>+</sup>) Hplc (Techogel15 C18, Gradient 2, water/acetonitrile/ TFA) rt 9.65 min.

**Example 22.**

10    **3-Amidino-benzoyl-L-phenylglycine 3-aminomethylbenzylamide**

MS TOF 416 (M+1<sup>+</sup>) Hplc (Techogel15 C18, Gradient 2, water/acetonitrile/ TFA) rt 10.26 min.

15    **Example 23.**

**3-Amidino-benzoyl-L-phenylglycine 4-aminomethylbenzylamide**

<sup>1</sup>H nmr (D<sub>2</sub>O) 8.10 (2H, m); 7.88 (1H, d, J=7.5Hz);  
7.65 (1H, t, J=7.5Hz); 7.4-7.1 (9H, m); 5.57 (1H, s);  
20    4.37 (2H, ABq); 4.06 (2H, s). MS TOF 416 (M+1<sup>+</sup>) Hplc  
(Techogel15 C18, Gradient 1, water/acetonitrile /TFA) rt  
12.34 min.

**Example 24.**

25    **3-Amidino-benzoyl-D-phenylglycine -4-(4-aminocyclohexyl methyl) cyclohexylamide**

<sup>1</sup>H nmr (D<sub>2</sub>O) 8.3-7.95 (3H, m); 7.85-7.6 (2H, m); 7.47  
(4H, m); 5.55 (1H, s); 3.59 (1H, m); 3.06 (1H, m); 2.1-  
1.5 (9H, m); 1.5-0.85 (11H, m). MS TOF 490 (M+1<sup>+</sup>). Hplc  
30    (Kromasil C4, Gradient 2, water/acetonitrile /TFA) rt  
18.30 min.

**Example 25.**

35    **3-Amidino-benzoyl-L-phenylglycine -4-(4-aminocyclohexyl methyl) cyclohexylamide**

<sup>1</sup>H nmr (D<sub>2</sub>O) 8.3-7.95 (3H, m); 7.85-7.6 (2H, m); 7.47  
(4H, m); 5.55 (1H, s); 3.59 (1H, m); 3.06 (1H, m); 2.1-

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1.5 (9H, m); 1.5-0.85 (11H, m). MS TOF 490 (M+1<sup>+</sup>). Hplc (Kromasil C4, Gradient 2, water/acetonitrile /TFA) rt 18.39 min.

5     **Example 26.**

**3-Amidino-benzoyl-D-phenylglyciny-D-2-naphthylalanine-5-aminopentamide**

<sup>1</sup>H nmr (d<sub>4</sub>methanol) δ 8.21 (1H, s); 8.14 (1H, d, J=7.5Hz); 7.96 (1H, d, J=7.5Hz); 7.80-7.65 (6H, m);  
10     7.45-7.15 (7H, m); 5.66 (1H, s); 4.56 (1H, m); 3.16 (2H, m); 3.02 (2H, m); 2.68 (2H, m); 1.45 (2H, m); 1.32 (2H, m); 1.12 (2H, m). MS TOF 579 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/TFA) rt 13.56 min.

15     **Example 27.**

**3-Amidino-benzoyl-D-phenylglyciny D-lysine-5-aminopentamide**

<sup>1</sup>H nmr (D<sub>2</sub>O) δ 8.10 (1H, s); 8.05 (1H, d, J=7.5Hz); 7.94 (1H, d, J=7.5Hz); 7.68 (1H, t, J=7.5Hz); 7.45 (5H, m);  
20     5.53 (1H, s); 4.22 (1H, t); 3.05 (2H, m); 2.90 (4H, dd); 1.8-1.0 (12H, m). MS TOF 510 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/TFA) rt 10.56 min.

**Example 28.**

25     **3-Amidino-benzoyl-D-phenylglycine-5-aminopentamide**

<sup>1</sup>H nmr (D<sub>2</sub>O) δ 7.85 (1H, s); 7.80 (1H, d, J=7.5Hz); 7.70 (1H, d, J=7.5Hz); 7.55 (1H, t, J=7.5Hz); 7.20 (5H, m); 5.30 (1H, s); 3.05 (2H, m); 2.60 (2H, m); 1.25 (4H, m); 0.90 (2H, m); MS TOF 382 (M+1<sup>+</sup>). Hplc (Jupiter5 C18,  
30     Gradient 2, water/acetonitrile/TFA) rt 11.26 min.

**Example 29.**

**3-Amidino-benzoyl-D-phenylglycine-R,S-3-aminopyrrolidinamide**

35     <sup>1</sup>H nmr (D<sub>2</sub>O) δ 8.18 (1H, s); 8.10 (1H, d); 7.88 (1H, d); 7.70 (1H, t); 7.50 (5H, m); 5.55 (1H, s); 4.50 (1H, m); 3 - 4 (4H, m); 2.33 (1H, m); 2.00 (1H, m). MS TOF

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366 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt 10.38 + 10.79 min.

**Example 30.**

5    **3-Amidino-benzoyl-D-phenylglycine 4-aminomethyl piperidinamide**

MS TOF 394 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt 11.40 min. (major) and 11.97 min. (minor).

10

**Method 2:** By solid phase strategy on a Protein Technologies, Symphony Multiple Peptide Synthesiser using Fmoc amino acids attached to Peg-2-chlorotrityl chloride resin: Typically the 2-chlorotrityl chloride resin was treated with a 2 fold excess of the Fmoc amino acid in a 1:1 mixture of DMF and dry DCM and DIPEA (2eq.). The resin was washed with DMF/DCM and deprotected with 20% piperidine in DMF before further modification. The resin was further modified by the attachment of acids. Activation of Fmoc protected amino acids (2-5eq) was by activation with TBTU/ DIPEA, all couplings ( minimum 120 min.) were carried out in DMF. Deprotection of the Fmoc group was achieved with 20% (v/v) piperidine in DMF. Other acid substituents were added as the HOBt or HOAt esters either by activation with HBTU/HATU or DIPCI with or without Boc protection of amino groups. Cleavage of the products from the resin was by treatment (30 min., ambient) with 10% (v/v) triethylsilane in TFA, filtration, evaporation and trituration with ether.

30

**Example 31.**

**3-Amidino-benzoyl-D-phenylglyciny-D-2-naphthylalaninyl-glycine**

35

Fmoc Glycine (0.2mmol, 59 mg.) in DMF (2ml) was added to 2 chlorotrityl chloride resin (1.0 mmol/g, 0.1g) pre

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swollen in dry DCM (2ml), then DIPEA (0.2 mmol). After 2h the resin was washed with DCM (6x5ml), DMF (6x5ml) and DCM (6x5ml). The resin was then air dried to allow aliquots to be taken for further modification.

5

On the Symphony Fmoc-Glycyl-2-chlorotrityl resin (0.1 mmol) was deprotected with 20% (v/v) piperidine in DMF and washed with DMF (6x 5ml) then treated with Fmoc-D-2-naphthylalanine (0.5 mmol 220 mg), DMF (2.5ml), TBTU in 10 DMF (1.25ml of a 450mM solution) and DIPEA in DMF (1.25ml of a 900 mM solution). The mixture was agitated with nitrogen for 2 hours. Deprotection and washing as above. The resin was then treated Fmoc-D-Phenylglycine (0.5 mmol 187 mg), DMF (2.5ml), TBTU in DMF (1.25ml of a 450mM 15 solution) and DIPEA in DMF (1.25ml of a 900 mM solution). The mixture was agitated with nitrogen for 2 hours. Deprotection and washing as above. HOBt (0.5 mmol, 68 mg) dissolved in DMF (4ml) was stirred at 0°C with DIPCI (0.5 mmol, 80µl), for 10 min. 20 3-amidinobenzoic acid TFA salt (0.5 mmol, 139 mg.) was added and stirring continued for 10 min. at room temperature. The mixture was transferred to the reaction vessel on the Symphony and agitated for 10 hours with nitrogen. The resin was then washed with DMF (6x5ml), 25 DCM (6x5ml) and air dried. The product was cleaved from the resin with 10% (v/v) triethylsilane in TFA (10ml) for 30 minutes, the resin filtered off, the TFA solution evaporated to dryness and triturated with diethyl ether to give the crude product. The crude product was then 30 dissolved in water (10ml), filtered and purified by preparative reverse phase Hplc..

<sup>1</sup>H nmr (DMSO) δ 8.15 (1H, s); 8.12 (1H, d, J=7.5Hz); 7.85 (1H, d, J=7.5Hz); 7.74 (5H, m); 7.65 (1H, t); 7.42 - 35 7.25 (7H, m); 5.73 (1H, s); 4.72 (1H, m); 3.75 (2H, d); signals at 3.3-3.0 obscured by solvent. MS TOF 552 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/TFA)

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rt 14.88 min.

Compounds made by the above method:-

5     **Example 32.**

**3-Amidino-benzoyl-D-phenylglyciny1-D-2-naphthylalaniny1  
N-methylglycine**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) mixture of rotomers, major product quoted.  
d 7.95 (2H, m); 7.87 (1H, d, J=7.5Hz); 7.71 (4H, m);  
10     7.62 (1H, t, J=7.5Hz); 7.36 (3H, m); 7.22 (5H, m); 5.57  
(1H, s); 5.19 (1H, dd); 3.95 (2H, ABq); 2.98 (3H, s).  
MS TOF 566 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1,  
water/acetonitrile/TFA) rt 14.86 min.

15     **Example 33.**

**3-Amidino-benzoyl-D-phenylglyciny1-D-2-naphthylalaniny1-  
D-proline**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) d 8.15 (1H, s); 8.08 (1H, d); 7.95 (1H,  
d); 7.82 (5H, m); 7.62 (1H, m); 7.22 - 7.5 (7H, m); 5.63  
20     (1H, s); 5.05 (1H, m); 4.35 (1H, m); 3.60 (2H, m);  
signals at 3.3 obscured by solvent; 3.1- (1H, m); 2.22,  
(1H, m); 2.0 (4H, m); . MS TOF 592 (M+1<sup>+</sup>). Hplc (Jupiter5  
C18, Gradient 1, water/acetonitrile/TFA) rt 15.32 min.

25     **Method 3:** By solid phase strategy on a Protein  
Technologies, Symphony Multiple Peptide Synthesiser  
using Fmoc amino acids attached to TentaGel S- resin  
(Rapp Polymere) via the Rink amide linker: Typically the  
TentaGel resin was treated with a 5 fold excess of the  
30     Rink linker, TBTU (1eq.), DIPEA (2eq.). The resin was  
washed with DMF and deprotected with 20% piperidine in  
DMF before further modification. The resin was further  
modified by the attachment of acids. Activation of Fmoc  
protected amino acid (2-5eq) was by TBTU/ DIPEA, all  
35     couplings ( minimum 120 min.) were carried out in DMF.  
Deprotection of the Fmoc group was achieved with 20%  
piperidine in DMF. Other acid substituents were added as



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the HOBt or HOAt esters either by activation with HBTU/HATU or DIPCI with or without Boc protection of amino groups. Cleavage of the products from the resin was by treatment (30 min., ambient) with 10% triethylsilane in TFA, filtration, evaporation and trituration with ether.

**Example 34.****3-Amidino-benzoyl-D-phenylglyciny l D-phenylalanine amide**

10

On the Symphony TentaGel S -NH<sub>2</sub> resin (0.1 mmol, 400 mg, 0.24 mmol./g) was treated with Rink linker (0.5 mmol, 270mg), DMF(2.5ml), TBTU in DMF(1.25ml of a 450mM solution) and DIPEA in DMF (1.25ml of a 900 mM solution). The mixture was agitated with nitrogen for 2 hours. Deprotection and washing as above.

15

On the Symphony Rink-TentaGel resin (0.1 mmol) was then treated with Fmoc-D-Phenylalanine- (0.5 mmol, 194mg), DMF(2.5ml), TBTU in DMF(1.25ml of a 450mM solution) and DIPEA in DMF (1.25ml of a 900 mM solution). The mixture was agitated with nitrogen for 2 hours. Deprotection and washing as above.

20

The resin (0.1 mmol) was then treated with Fmoc-D-Phenylglycine (0.5 mmol, 187mg), DMF(2.5ml), TBTU in DMF(1.25ml of a 450mM solution) and DIPEA in DMF (1.25ml of a 900 mM solution). The mixture was agitated with nitrogen for 2 hours. Deprotection and washing as above.

25

HOBt (0.5 mmol, 68 mg) dissolved in DMF(4ml) was stirred at ice bath with DIPCI (0.5 mmol, 80 $\mu$ l) for 10 min. 3-amidino-benzoic acid TFA salt (0.5 mmol, 139 mg.) was added and stirring continued for 10 min. at room temperature. The mixture was then transferred to the reaction vessel on the Symphony and agitated for 10 hours with nitrogen. The resin was then washed with DMF (6x5ml), DCM (6x5ml) and air dried. The product was

30

35

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cleaved from the resin with 10% triethylsilane in TFA (10ml) for 30 minutes, the resin filtered off, the TFA solution evaporated to dryness and triturated with diethyl ether to give the crude product. The crude product was then dissolved in water (10ml), filtered and purified by preparative reverse phase Hplc.

<sup>1</sup>H nmr (d<sub>2</sub>methanol) δ 8.18 (1H, s); 8.10 (1H, d, J=7.5Hz); 7.87 (1H, d, J=7.5Hz); 7.72 (1H, t, J=7.5Hz); 7.40 (5H, m); 7.10 (5H, m); 5.53 (1H, s); 4.55 (dd, partially obscured by solvent); 3.08 (1H, dd); 2.87 (1H, dd). MS TOF 444 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/TFA) rt 11.70 min.

Compounds made by the above method:-

**Example 35.**

**3-Amidino-benzoyl-D-phenylglyciny-L-asparagine amide**

<sup>1</sup>H nmr (D<sub>2</sub>O) δ 8.10 (1H, s); 8.04 (1H, d, J=7.5Hz); 7.91 (1H, d, J=7.5Hz); 7.66 (1H, t, J=7.5Hz); 7.42 (5H, m); 5.47 (1H, s); 4.73 (m, partially obscured by solvent); 2.71 (2H, m); 2.59 (2H, m). MS TOF 411 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt 10.49 min.

**Example 36.**

**3-Amidino-benzoyl-D-phenylglyciny-D-2-naphthylalanine amide**

<sup>1</sup>H nmr (d<sub>2</sub>methanol) δ 8.18 (1H, s); 8.12 (1H, d, J=7.5Hz); 7.95 (1H, d, J=7.5Hz); 7.74 (5H, m); 7.42 (3H, m); 7.25 (5H, m); 5.63 (1H, s); 3.12 (1H, m); signals at ~4.8 and 3.3 obscured by solvent. MS TOF 494 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/TFA) rt 15.46 min.

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**Example 37.****3-Amidino-benzoyl-D-phenylglyciny-D-valine amide**

<sup>1</sup>H nmr (d<sub>4</sub>methanol) δ 8.44 (1H, s); 8.36 (1H, d, J=7.5Hz); 8.10 (1H, d, J=7.5Hz); 7.86 (1H, t, J=7.5Hz);  
5 7.70 (2H, m); 7.55 (3H, m); 5.96 (1H, s); 4.40 (1H d);  
2.21 (1H, m); 1.13 (6H, t). ). MS TOF 396 (M+1<sup>+</sup>). Hplc  
(Jupiter5 C18, Gradient 1, water/acetonitrile/TFA) rt  
5.71 min.

10 **Example 38.****3-Amidino-benzoyl-D-phenylglyciny-D-lysine amide**

<sup>1</sup>H nmr (D<sub>2</sub>O) δ 8.10 (1H, s); 8.04 (1H, d, J=7.5Hz); 7.95  
(1H, d, J=7.5Hz); 7.66 (1H, t, J=7.5Hz); 7.43 (5H, m);  
5.56 (1H, s); 4.26 (1H, m); 2.91 (2H, t); 1.77 (2H, m);  
15 1.62 (2H, m); 1.42 (2H, m). MS TOF 425 (M+1<sup>+</sup>). Hplc  
(Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt  
10.73 min.

**Example 39.**20 **3-Amidino-benzoyl-D-phenylglyciny-L-lysine amide**

<sup>1</sup>H nmr (D<sub>2</sub>O) δ 8.08 (1H, s); 8.00 (1H, d, J=7.5Hz); 7.99  
(1H, d, J=7.5Hz); 7.61 (1H, t, J=7.5Hz); 7.40 (5H, m);  
5.52 (1H, s); 4.21 (1H, m); 2.68 (2H, m); 1.74 (1H, m);  
1.60 (1H, m); 1.40 (2H, m); 1.08 (1H, m). MS TOF 425  
25 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2,  
water/acetonitrile/TFA) rt 10.61 min.

**Example 40.**30 **3-Amidino-benzoyl-D-phenylglyciny-D,L-phenylglyciny-L-valine amide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) mixture of diastereomers δ 8.39 (1H, m);  
8.15 (1H, m); 7.93 (1H, m); 7.70 (1H, m); 7.41 (10H, m);  
5.71 (1H, d); 5.46 (1H, m); 4.14 (1H, m); 2.77 (1H, m);  
0.97 (3H, m); 0.65 (3H, m). MS TOF 529 (M+1<sup>+</sup>). Hplc  
35 (Jupiter5 C18, Gradient 1, water/acetonitrile/TFA) rt  
13.10 min.

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**Example 41.****3-Amidino-benzoyl-D-phenylglycinyll-D-asparagine-D-asparagine amide**

<sup>1</sup>H nmr (D<sub>2</sub>O) δ 8.28 (1H, s); 8.20 (1H, d, J=7.5Hz); 8.08 (1H, d, J=7.5Hz); 7.82 (1H, t, J=7.5Hz); 7.59 (5H, m); 5.72 (1H, s); 4.70 (1H, dd); 2.85 (4H, m). MS TOF 526 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt 11.14 min.

10 **Example 42.****3-Amidino-benzoyl-D-phenylglycinyll-D-leucine amide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) δ 8.23 (1H, s); 8.14 (1H, d, J=7.5Hz); 7.93 (1H, d, J=7.5Hz); 7.72 (1H, t, J=7.5Hz); 7.48 (5H, m); 5.62 (1H, s); 4.32 (1H, m); 1.58 (2H, m); 0.87 (6H, dd). MS TOF 410 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt 14.98 min.

**Example 43.****3-Amidino-benzoyl-D-phenylglycinyll-L-phenylalanine amide**

20 <sup>1</sup>H nmr (CD<sub>3</sub>CN) δ 8.20 (1H, s); 8.14 (1H, d, J=7.5Hz); 7.96 (1H, d, J=7.5Hz); 7.72 (1H, t, J=7.5Hz); 7.35 (5H, m); 7.14 (3H, m); 7.04 (2H, m); 5.60 (1H, s); 5.46 (1H, dd); 3.15 (1H, dd); 2.80 (1H, m). MS TOF 444 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/TFA) rt 25 12.78 min.

**Example 44.****3-Amidino-benzoyl-D-phenylglycinyll-L-2-naphthylalanine amide**

30 <sup>1</sup>H nmr (d<sub>4</sub>methanol) δ 8.26 (1H, s); 8.19 (1H, d, J=7.5Hz); 7.95 (1H, d, J=7.5Hz); 7.80 (1H, m); 7.67 (3H, m); 7.53 (1H, s); 7.45 (2H, m); 7.25 (1H, d); 7.08 (3H, m); 6.92 (2H, t); 5.61 (1H, s); 3.45 (1H, m); 3.0 (1H, m) signal at ~4.8 obscured by solvent. MS TOF 494 (M+1<sup>+</sup>).  
35 Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/TFA) rt 16.17 min.

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**Example 45.****3-Amidino-benzoyl-D-phenylglycinyll-L-valine amide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) d 8.17 (1H, s); 8.08 (1H, d, J=7.5Hz);  
7.89 (1H, d, J=7.5Hz); 7.65 (1H, t, J=7.5Hz); 7.37 (5H,  
5 m); 5.64 (1H, s); 4.08 (1H d); 1.97 (m, partially  
obscured by solvent); 0.63 (6H, t). MS TOF 396 (M+1<sup>+</sup>).  
Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA)  
rt 9.34 min.

10 **Example 46.****3-Amidino-benzoyl-D-phenylglycinyll-D-asparagine amide**

<sup>1</sup>H nmr (D<sub>2</sub>O) d 7.91 (1H, s); 7.86 (1H, d, J=7.5Hz); 7.63  
(1H, d, J=7.5Hz); 7.48 (1H, t, J=7.5Hz); 7.23 (5H, m);  
5.30 (1H, s); 4.42 (m, partially obscured by solvent);  
15 2.57 (2H, m). MS TOF 411 (M+1<sup>+</sup>). Hplc (Jupiter5 C18,  
Gradient 2, water/acetonitrile/TFA) rt 10.23 min.

**Example 47.**20 **3-Amidino-benzoyl-L-phenylglycinyll-D-2-naphthylalanine  
amide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN/D<sub>2</sub>O) 8.17 (1H, s); 8.10 (1H, d); 7.92 (1H,  
d); 7.81 (1H, m); 7.67 (3H, m); 7.53 (1H, s); 7.48 (2H,  
m); 7.22 (1H, d); 7.12 (3H, m); 6.98 (2H, m); 5.56  
(1H,s); 4.70 (1H, dd); 3.35 (1H, dd); 3.00 (1H,dd). MS  
25 TOF 494 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1,  
water/acetonitrile/TFA) rt 15.46 min.

**Example 48.**30 **3-Amidino-benzoyl-L-phenylglycinyll-L-2-naphthylalanine  
amide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.07 (1H, s); 8.01 (1H, d); 7.92 (1H, d);  
7.78 (1H, m); 7.66 (2H, s); 7.41 (4H, m); 7.14 (6H, d);  
5.46 (1H,s); 4.73 (1H, dd); 3.38 (1H, dd); 3.08 (1H,dd).  
MS TOF 494 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1,  
35 water/acetonitrile/TFA) rt 14.51 min.

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**Example 49.****3-Amidino-benzoyl-D-phenylglyciny-D-4-chloro  
phenylalanine amide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) δ 8.39 (1H, s); 8.32 (1H, d, J=7.5Hz);  
5 8.13 (1H, d, J=7.5Hz); 7.90 (1H, t, J=7.5Hz); 7.51 (5H,  
m); 7.40 (4H, ABq); 5.70 (1H, s); 4.77 (1H, dd); 3.36  
(1H, dd); 3.09 (1H, dd). MS TOF 479 (M+1<sup>+</sup>). Hplc  
(Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt  
13.63 min.

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**Example 50.****3-Amidino-benzoyl-D-phenylglyciny-D-isoleucine amide**

MS TOF 410 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2,  
water/acetonitrile/TFA) rt 14.36 min.

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**Example 51.****3-Amidino-benzoyl-D-phenylglyciny-D-tyrosine amide**

MS TOF 460 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2,  
water/acetonitrile/TFA) rt 12.56 min.

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**Example 52.****3-Amidino-benzoyl-D-phenylglyciny-D-1-naphthylalanine  
amide**

MS TOF 494 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2,  
25 water/acetonitrile/TFA) rt 22.19 min.

**Example 53.****3-Amidino-benzoyl-D-phenylglyciny-D-threonine amide**

MS TOF 398 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2,  
30 water/acetonitrile/TFA) rt 10.63 min.

**Example 54.****3-Amidino-benzoyl-D-phenylglyciny-D-histidine amide**

MS TOF 434 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2,  
35 water/acetonitrile/TFA) rt 10.04 min.

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**Example 55.**

3-Amidino-benzoyl-D-phenylglyciny1-D-phenylalanine amide  
MS 444 TOF (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2,  
water/acetonitrile/TFA) rt 14.82 min.

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**Example 56.**

3-Amidino-benzoyl-D-phenylglyciny1-D-phenylalaniny1 D-  
proline amide  
MS TOF 541 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2,  
water/acetonitrile/TFA) rt 11.54 min.

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**Example 57.**

3-Amidino-benzoyl-D-phenylglyciny1-D-tryptophan amide  
MS TOF 483 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2,  
water/acetonitrile/TFA) rt 17.14 min.

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Method 4: By solution phase strategy: Typically an  
activated Boc-amino acid was treated with an amine  
(primary or secondary) or alcohol (1eq.). Activation of  
Boc protected amino acid was by HATU or TBTU/  
DIPEA(1:2), all couplings (minimum 120 min.) were  
carried out in DMF. After an aqueous work up the  
deprotection of the Boc group was achieved with TFA.  
Other acid substituents were added as the HOBT or HOAT  
esters either by activation with HBTU/HATU or DIPCI with  
or without Boc protection of amino groups. The final  
products were purified by preparative reverse phase  
Hplc.

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**Example 58.**

3-Amidino-benzoyl-D-phenylglycine -1-adamantylamide

Boc D-phenylglycine (251 mg, 1 mmol.) was dissolved in  
DMF(3ml) with HATU (380 mg., 1 mmol.) and DIPEA(350μl .,  
2 mmol.). To this mixture was added 1-adamantylamine  
hydrochloride(187 mg., 1 mmol.) and DIPEA (170μl., 1  
mmol.). The mixture was stirred overnight. The mixture

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was then taken up into ethylacetate and washed with water, sodium carbonate solution, water, 10% hydrochloric acid solution and water. The ethylacetate was evaporated without drying and treated immediately with TFA for 30 min. The TFA was then evaporated to dryness and the product triturated with diethylether. TEA(1ml) was added and evaporated to dryness. HOBt (1 mmol, 136 mg) dissolved in DMF(4ml) was stirred at 0°C and treated with DIPCI (1 mmol, 160 $\mu$ l), for 10 min. 3 amidino benzoic acid TFA salt (1 mmol., 278 mg.) was added and stirring continued for 10 min. at room temperature. The mixture was then added to the D-phenylglycine adamantyl amide and stirred overnight. The crude product was dissolved in water/acetonitrile (20ml), filtered and purified by preparative Hplc to yield pure product (150 mg.)

<sup>1</sup>H nmr (D<sub>2</sub>O/CD<sub>3</sub>CN) 8.21 (1H, s); 8.13 (1H, d, J=7.5Hz); 7.92 (1H, d, J=7.5Hz); 7.70 (1H, t, J=7.5Hz); 7.46 (2H, m); 7.34 (3H, m); 5.52 (1H, s); 2.15-1.52 (15H, m). M.S. TOF 431 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/ TFA) rt 19.54 min.

Compounds made by the above method:-

**Example 59.**

**3-Amidino-benzoyl-D-phenylglycine -1-adamantylmethanamide**

<sup>1</sup>H nmr (D<sub>2</sub>O/CD<sub>3</sub>CN) 8.23 (1H, s); 8.15 (1H, d, J=7.5Hz); 7.85 (1H, d, J=7.5Hz); 7.71 (1H, t, J=7.5Hz); 7.46 (2H, m); 7.35 (3H, m); 5.59 (1H, d); 3.0 (2H, s obscured by solvent); 2.15-1.52 (15H, m). M.S. TOF 445 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/ TFA) rt 21.35 min.



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**Example 60.****3-Amidino-benzoyl-D-phenylglycine-2-phenylethylamide**

<sup>1</sup>H nmr (d<sub>4</sub> methanol) 8.18 (2H, m); 7.87 (1H, d, J=7.5Hz);  
7.60 (1H, t, J=7.5Hz); 7.30 (5H, m); 7.02 (5H, m); 5.53  
5 (1H, s); 3.32 (1H, m); 1H signal at ~3.25 obscured by  
solvent); 2.68 (2H, t). M.S. TOF 401 (M+1<sup>+</sup>). Hplc  
(Kromasil C4, Gradient 1, water/acetonitrile/TFA) rt  
15.56 min.

10 **Example 61.****3-Amidino-benzoyl-D-phenylglycine-2-(1-adamantyl) ethyl ester**

<sup>1</sup>H nmr (d<sub>4</sub> methanol) 8.27 (2H, m); 7.94 (1H, d J=7.5Hz);  
7.72 (1H, t, J=7.5Hz); 7.42 (5H, m); 5.66 (1H, s); 4.25  
15 (2H, m); 1.82 (3H, s); 1.61 (6H, m); 1.38 (8H, m). M.S.  
TOF 459 (M+1<sup>+</sup>). Hplc (Kromasil C4, Gradient 1,  
water/acetonitrile/ TFA) rt 23.17 min.

**Example 62.**20 **3-Amidino-benzoyl-D-phenylglycine-2-methylbenzylamide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.14 (2H, m); 7.88 (1H, d, J=7.5Hz); 7.65  
(1H, t, J=7.5Hz); 7.45 (2H., m); 7.35 (3H, m); 7.04 (4H,  
m); 5.59 (1H, s); 4.30 (2H, AB q); 2.13 (3H, s). M.S.  
TOF 401 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1,  
25 water/acetonitrile/ TFA) rt 14.81 min.

**Example 63.****3-Amidino-benzoyl-D-phenylglycine-4-benzoyl piperidinamide**

30 <sup>1</sup>H nmr (DMSO) 8.40 (2H, m); 8.10 (1H, d); 7.70 (1H, t);  
7.50 (10H, m); 5.55 (1H, s); 3.60 (1H, m); 2.5 (2H,  
m); .1.00, (6H, m); .S. TOF 469 (M+1<sup>+</sup>). Hplc (Jupiter5 C18,  
Gradient 1, water/acetonitrile/ TFA) rt 11.43 min.

35 **Example 64.****3-Amidino-benzoyl-D-phenylglycine-4-methylbenzylamide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.30 (1H, s); 8.22 (1H, d, J=7.5Hz); 7.95

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(1H, d, J=7.5Hz); 7.71(1H, t, J=7.5Hz); 7.51 (2H., m); 7.34 (3H, m); 7.04 (4H, s); 5.76 (1H, s); 4.30 (2H, AB q); 2.30 (3H, s). M.S. TOF 401 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/ TFA) rt 15.20 min.

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**Example 65.****3-Amidino-benzoyl-D-phenylglycine-1-naphthylmethanamide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN/D<sub>2</sub>O) 8.14 (1H, s); 8.02 (1H, d, J=7.5Hz); 7.84 (3H, m); 7.60 (1H, d, J=7.5Hz); 7.32 (5H, m, J=7.5Hz); 7.12 (5H., m); 5.58 (1H, s); 4.50 (2H, AB q); 3.03 (2H, s). M.S. TOF 436 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/ TFA) rt 16.77 min.

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**Example 66.****3-Amidino-benzoyl-D-phenylglycine-1-D-phenylalanine -N-pyrrolidinamide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN/D<sub>2</sub>O) 8.14 (1H, s); 8.03 (1H, d, J=7.5Hz); 7.94 (1H, d, J=7.5Hz); 7.70(1H, t, J=7.5Hz); 7.42 (5H, m); 7.42 (5H., m); 5.62 (1H, s); 4.80 (1H, t); 3.40 , (1H, m); 3.20 (2H, m); 3.02 , (2H, d); 2.80 , (1H, m); 1.65 , (4H, m); . M.S. TOF 499 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/ TFA) rt 14.03 min.

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**Example 67.****3-Amidino-benzoyl-D-phenylglycine-3-methylbenzylamide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 7.95 (1H, s); 7.81 (1H, d, J=7.5Hz); 7.60 (1H, d, J=7.5Hz); 7.45 (1H, t, J=7.5Hz); 7.23 (2H., m); 7.12 (3H, m); 6.60-6.75 (4H, m); 5.40 (1H, s); 4.10 (2H, AB q); 1.95 (3H, s). M.S. TOF 401 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/ TFA) rt 14.52 min.

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**Example 68.****3-Amidino-benzoyl-D-phenylglycine-2adamantylamide**

MS TOF 431 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 12.68 min.

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**Method 5:** By solid phase strategy using Boc amino acids

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attached to Kaiser Oxime resin (Novabiochem): Typically the resin is treated with a 2 fold excess of the Boc phenylglycine symmetrical anhydride. The resin was washed with DCM/DMF then DCM and deprotected with 25%TFA in DCM before further modification. The resin was further modified by the attachment of acids. Activation of Boc protected amino acid (2-5eq) was by TBTU/ DIPEA, all couplings (minimum 120 min.) were carried out in DCM/DMF. Deprotection of the Boc group was achieved with 25%TFA in DCM. Other acid substituents were added as the HOBt or HOAt esters either by activation with HBTU/HATU or DIPCI with or without Boc protection of amino groups. Cleavage of the products from the resin was by treatment (1 day, ambient) with an amine or alcohol in chloroform, washing with chloroform and methanol/chloroform.

**Example 69.****3-Amidino-benzoyl-D-phenylglycine cyclohexyl amide**

Boc-D-Phenylglycine (0.5 mmol, 126 mg) was dissolved in DCM (5ml) and treated with DIPCI (0.5 mmol., 80 $\mu$ l) and stirred for 10 min. A white precipitate forms which was dissolved by adding DMF. This mixture was added to Oxime resin (0.11 mmol, 330 mg, 0.33 mmol./g) previously swelled with DCM. 4-Dimethyl amino pyridine (10 mg, cat.) was added and the mixture swirled for 2 hours. The resin was filtered and washed with DMF/DCM then DCM. The resin was then treated with 25% TFA/DCM (20ml) for 20 min., filtered and washed with DCM. HOBt (0.5 mmol, 68 mg) dissolved in DMF(4ml) was stirred at 0°C with DIPCI (0.5 mmol, 80 $\mu$ l) for 10 min. 3 amidino-benzoic acid TFA salt (0.5 mmol, 139 mg.) was added and stirring continued for 10 min. at room temperature. The mixture was then added to the phenylglycine-oxime resin and agitated for 2 hours. The resin was filtered washed with DMF, DCM and chloroform

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then treated with cyclohexylamine (0.3 mmol, 40  $\mu$ l) in chloroform (2ml) and agitated for 1 day. The resin was then filtered off and washed with chloroform (10ml) and chloroform/methanol (1:1, 10ml). The combined organic  
5 extracts were evaporated to dryness and washed with 10% aqueous acetic acid (5-10ml). The aqueous insoluble residue was dissolved in acetonitrile/water and lyophilised.  
MS TOF 379 ( $M+1^+$ ). Hplc (Jupiter5 C18, Gradient 3,  
10 water/acetonitrile/TFA) rt 10.62 min.

**Example 70.**

**3-Amidino-benzoyl-D-phenylglycine-3,3-dimethylbutylamide**  
MS TOF 382 ( $M+1^+$ ). Hplc (Jupiter5 C18, Gradient 3,  
15 water/acetonitrile/TFA) rt 13.90 min.

**Example 71.**

**3-Amidino-benzoyl-D-phenylglycine-1,2,3,4-tetrahydro-1-naphthylamide**  
20 MS TOF 427 ( $M+1^+$ ). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 11.74 min.

**Example 72.**

**3-Amidino-benzoyl-D-phenylglycine-R,S 3-methyl-2-butylamide**  
25 MS TOF 367 ( $M+1^+$ ). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 10.21 + 10.42 min.

**Example 73.**

**3-Amidino-benzoyl-D-phenylglycine-3-phenylpropylamide**  
30 MS TOF 415 ( $M+1^+$ ). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 14.00 min.

**Example 74.**

**3-Amidino-benzoyl-D-phenylglycine-3-trifluoromethyl benzylamide**  
35 MS TOF 455 ( $M+1^+$ ). Hplc (Jupiter5 C18, Gradient 3,

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water/acetonitrile/TFA) rt 14.32 min.

**Example 75.**

**3-Amidino-benzoyl-D-phenylglycine-3-fluorobenzylamide**

5 MS TOF 405 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 12.56 min.

**Example 76.**

**3-Amidino-benzoyl-D-phenylglycine-2-methyl propylamide**

10 MS TOF 353 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 11.10 min.

**Example 77.**

**3-Amidino-benzoyl-D-phenylglycine-4-phenyl butylamide**

15 MS TOF 429 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 14.96 min.

**Example 78.**

**3-Amidino-benzoyl-D-phenylglycine-cyclohexylmethanamide**

20 MS TOF 493 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 11.52 min.

**Example 79.**

**3-Amidino-benzoyl-D-phenylglycine-2,2 diphenylethylamide**

25 MS TOF 478 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 12.65 min.

**Example 80.**

**3-Amidino-benzoyl-D-phenylglycine-R-1-(1-naphthyl)ethanamide**

30 MS TOF 450 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 12.26 min.

**Example 81.**

**3-Amidino-benzoyl-D-phenylglycine-S-1-(2-naphthyl)ethanamide**

35 MS TOF 450 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,

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water/acetonitrile/TFA) rt 11.19 min.

**Method 6:** By solution phase strategy: Typically an activated Fmoc amino acid was treated with an alcohol (1eq.) which contains a Boc protected amino group. Activation of Fmoc protected amino acid was by HATU or TBTU/ DIPEA(1:2), all couplings (minimum 120 min.) were carried out in DMF with DBU as catalyst After an aqueous work up the deprotection of the Fmoc group was achieved with 20% piperidine in DMF followed by evaporation and column chromatography. Other acid substituents were added as the HOBt or HOAt esters either by activation with HBTU/HATU or DIPCI with or without Boc protection of amino groups. The products were purified by preparative reverse phase Hplc, followed by deprotection of the amino groups with TFA. Further modification to acetamidine or amidine can be achieved by known literature methods.(2,3)

**Example 82.**

**3-Amidino-benzoyl-D-phenylglycine -(4-piperidinyl)methyl ester**

Fmoc D-Phenylglycine( 1 mmol., 374 mg) was dissolved in DMF(5ml) with HATU (1 mmol., 380 mg) and DIPEA(2 mmol., 350 $\mu$ l). To this mixture was added N-Boc 4-hydroxymethyl piperidine (215 mg, 1 mmol.) and DBU (100 $\mu$ l). The mixture was stirred overnight. The mixture was then taken up into ethylacetate(50ml) and washed with water, sodium carbonate solution, water, 10% hydrochloric acid solution and water. The ethylacetate layer was dried over magnesium sulphate, filtered and evaporated. The product was then treated with 20% piperidine in DMF for 90 min. this was then evaporated to dryness and then dissolved in methanol and adsorbed onto silica gel 60. The material was then chromatographed on silica gel 60, eluting first with hexane/ethylacetate (2:1) then

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hexane/ethylacetate (1:2), the required product eluting in the second solvent. The combined fractions were evaporated to dryness.

5 HOBt (1 mmol, 136 mg) dissolved in DMF(4ml) was stirred at 0°C with DIPCI (1 mmol, 160 $\mu$ l) for 10 min. 3 amidino-benzoic acid TFA salt (1 mmol., 278 mg.) was added and stirring continued for 10 min. at room temperature. The mixture was then added to the D-phenylglycine N-Boc-4-piperidine methyl ester and 10 stirred overnight. The mixture was evaporated to dryness on high vacuum, water and acetonitrile added to allow full dissolution and the whole lyophilised. The resulting crude product was treated with TFA/water (9:1) for 30 min. and evaporated to dryness. The crude product 15 was dissolved in water/acetonitrile (20ml), filtered and purified by preparative reverse phase Hplc. Producing pure product (200mg).

<sup>1</sup>H nmr (D<sub>2</sub>O) 8.12 , (1H,m); 8.05 (1H, d); 7.90 (1H, d); 20 7.65 (1H, t); 7.45 (5H, m); 5.65 , (1H,s); 4.10 , (2H,d); 3.30 (2H,m); 2.80 (2H, m); 1.90 (1H, m); 1.75 (2H, m); 1.30 (2H, m); MS TOF 395 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt 12.48 min.

25 Compounds made by the above method:-

#### Example 83

#### 3-Amidino-benzoyl-D-phenylglycine-2-(4-piperidinyl) ethyl ester

30 <sup>1</sup>H nmr (D<sub>2</sub>O) 8.12 , (1H,m); 8.05 (1H, d); 7.90 (1H, d); 7.65 (1H, t); 7.45 (5H, m); 5.65 , (1H,s); 4.25 , (2H,m); 3.20 (2H,m); 2.60 (2H, m); 1.65 (2H, m); 1.50 (2H, m); 1.20 (3H, m); MS TOF 409 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt 13.14 min.

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**Example 84.****3-Amidino-benzoyl-D-phenylglycine-2-(N-acetimino-4-piperidinyl) ethyl ester**

<sup>1</sup>H nmr (D<sub>2</sub>O) 8.22 , (1H,m); 8.20 (1H, d); 8.02 (1H, d);  
5 7.80 (1H, t); 7.60 (5H, m); 5.75 , (1H,s); 4.35 , (2H,m);  
3.85 (2H,m); 3.00 (3H, m); 2.30 (3H,s); 1.65 (3H, m);  
1.50 (1H, m); 1.20 (2H, m); MS TOF 450 (M+1<sup>+</sup>). Hplc  
(Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt  
14.92 min.

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**Example 85.****3-Amidino-benzoyl-D-phenylglycine-(N-acetimino-4-piperidinyl) methyl ester**

<sup>1</sup>H nmr (D<sub>2</sub>O) 8.12 , (1H,m); 8.05 (1H, d); 7.90 (1H, d);  
15 7.65 (1H, t); 7.40 (5H, m); 5.60 , (1H,s); 4.05 , (2H,m);  
3.75 (2H,m); 3.10 (1H, m); 2.10 (3H,d); 1.90 (1H, m);  
1.60 (2H, m); 1.20 (2H, m); 0.90 (1H,m); MS TOF 436  
(M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2,  
water/acetonitrile/TFA) rt 13.34 min.

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**Example 86.****3-Amidino-benzoyl-D-phenylglycine-(N-amidino-4-piperidinyl) methyl ester**

<sup>1</sup>H nmr (D<sub>2</sub>O) 8.02 , (1H,m); 7.97 (1H, d); 7.80 (1H, d);  
25 7.55 (1H, t); 7.40 (5H, m); 5.55 , (1H,s); 4.05 , (2H,m);  
3.65 (2H,m); 2.80 (2H, m); 1.80 (1H,m); 1.50 (2H, m);  
1.10 (1H, m); 0.80 (1H,m); MS TOF 437 (M+1<sup>+</sup>). Hplc  
(Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt  
13.61 min.

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**Example 87.****3-Amidino-benzoyl-D-phenylglycine-2-(N-amidino-4-piperidinyl) ethyl ester**

<sup>1</sup>H nmr (D<sub>2</sub>O) 8.17 , (1H,m); 8.07 (1H, d); 7.93 (1H, d);  
35 7.70 (1H, t); 7.45 (5H, m); 5.60 , (1H,s); 4.25 , (2H,m);  
3.55 (2H,m); 2.75 (2H, m); 1.60 (4H,m); 1.25 (1H, m);  
1.00 (2H, m); MS TOF 451 (M+1<sup>+</sup>). Hplc (Jupiter5 C18,



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Gradient 2, water/acetonitrile/TFA) rt 14.97 min.

**Example 88.**

**2-Hydroxy-5-amidino-benzoyl D-phenylglycine-4-aminomethylcyclohexyl methylamide**

5-Bromosalicylic acid (9.31mmol., 2.02g) was dissolved in DMF (4.5ml) and treated with copper (I) cyanide (11.33 mmol., 1.2eq., 1.015g) and heated under reflux for 4 hours. The solution was allowed to cool and poured into a solution of ferric chloride (2g) and concentrated hydrochloric acid (0.7ml) in water (3ml). The mixture was heated for 10 min. at 60°C, then cooled and filtered, the solid was washed with water, then recrystallised ethanol/water. Product 0.73g, 48%.

On the Symphony bis-1,4-aminomethylcyclohexane 2 chlorotriptyl chloride resin (0.1 mmol) (See method 1) was treated with Fmoc-D-Phenylglycine (0.5 mmol, 187mg), DMF(2.5ml), TBTU in DMF(1.25ml of a 450mM solution) and DIPEA in DMF (1.25ml of a 900 mM solution) and agitated with nitrogen for 2 hours. Deprotection and washing as above.

HOBt (0.5 mmol, 68 mg) dissolved in DMF(4ml) was stirred at 0°C with DIPCI (0.5 mmol, 80µl) for 10 min. 2-hydroxy-5-cyanobenzoic acid (0.5 mmol, 84 mg.) was added and stirring continued for 10 min. at room temperature. The mixture was then transferred to the reaction vessel on the Symphony and agitated for 10 hours with nitrogen. The resin was washed with DMF (6x5ml), DCM (6x5ml) and air dried. The product was cleaved from the resin with 10% triethylsilane in TFA (10ml) for 30 minutes, the resin filtered off, the TFA solution evaporated to dryness and triturated with diethyl ether to give the crude product. The crude product was dissolved in saturated hydrogen chloride in ethanol (10ml), sealed and allowed to stand overnight. The solution was

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evaporated to dryness and treated with saturated ammonia in ethanol (10ml) and allowed to stand overnight. The solution was then evaporated to dryness, dissolved in water and purified by reverse phase Hplc.

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<sup>1</sup>H nmr (D<sub>2</sub>O), 8.35 , (1H,s); 7.90 , (1H,d); 7.55 , (5H,m); 7.25 , (1H,d); 5.65 (1H, s); 3.20 (2H, m); 2.90 (2H, m); 1.40-1.80 (7H, m); 0.95 (3H, m) MS TOF 438 (M+1<sup>+</sup>). Hplc (Techogel5 C18, Gradient 2, water/acetonitrile/TFA) rt 14.11 min.

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**Example 89.****2-Amino-5-amidinobenzoyl-D-phenylglycine-4-aminomethylcyclohexyl methylamide**

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2-Amino-5-bromo benzoic acid (1.6 mmol., 345mg) was dissolved in N-methylpyrrolidone (5ml) and treated with copper (I) cyanide (2.39 mmol., 1.5eq., 207mg) under reflux for 4.5 hours. The solution was allowed to cool and poured into water (10ml). The mixture was acidified to pH3 with hydrochloric acid and extracted with ethylacetate, the ethylacetate was washed with water, dried over magnesium sulphate and evaporated to a yellow solid (145mg, 56%).

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On the Symphony bis-1,4-aminomethylcyclohexane-2-chlorotriptyl resin (0.1 mmol) (See method 1) was treated with Fmoc-D-Phenylglycine (0.5 mmol, 187 mg), DMF (2.5ml), TBTU in DMF (1.25ml of a 450mM solution) and DIPEA in DMF (1.25ml of a 900 mM solution) for 2 hours. Deprotection and washing as above.

30

HOBt (0.5 mmol, 68 mg) dissolved in DMF (4ml) was stirred at 0°C with DIPCI (0.5 mmol, 80μl) for 10 min. 2-amino-5-cyanobenzoic acid (0.5 mmol, 84 mg.) was added and stirring continued for 10 min. at room temperature. The mixture was transferred to the reaction vessel on the Symphony and agitated for 10 hours with nitrogen. The

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resin was then washed with DMF (6x5ml), DCM (6x5ml) and air dried. The product was cleaved from the resin with 10% triethylsilane in TFA (10ml) for 30 minutes, the resin filtered off, the TFA solution evaporated to dryness and triturated with diethyl ether to give the crude product. The crude product was then dissolved in saturated hydrogen chloride in ethanol (10ml), sealed and allowed to stand overnight. The solution was then evaporated to dryness and treated with saturated ammonia in ethanol (10ml) and allowed to stand overnight. The solution was then evaporated to dryness, dissolved in water and purified by reverse phase Hplc.

<sup>1</sup>H nmr (D<sub>2</sub>O) Mixture of cyclohexyl cis and trans isomers 7.80 (1H, s); 7.50 (1H, d, J=7.5Hz); 7.35 (5H, m); 6.75 (1H, d, J=7.5Hz); 5.35 (1H, s); 2.87 (2H, m); 2.60 (2H, m); 1.00-1.60 (7H, m); 0.70 (3H, m) MS TOF 437 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt 13.11 min. (major), 13.31 (minor).

20

Prepared by Method 2

**Example 90.**

**3-Amidino-benzoyl-D-phenylglyciny-D-2-naphthylalanine**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.00 (1H, s); 7.95 (1H, d); 7.87 (1H, d); 7.72 (4H, m); 7.63 (2H, m); 7.38 (3H, m); 7.25 (4H, m); 5.60 (1H, s); 4.70 (1H, m); 3.25 (2H, m). MS TOF 495 (M+1<sup>+</sup>). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 10.19 min.

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**Example 91.**

**3-Amidino-benzoyl-D-phenylglycine-1,2,3,4, tetrahydroisoquinolin-2-yl-3-carboxylic acid**

<sup>1</sup>H nmr (DMSO) mixture of isomers and rotomers (only one isomer cited in nmr) 8.28 (1H, s); 8.18 (1H, d); 7.90 (1H, d); 7.69 (1H, t); 7.35 (4H, m); 7.15 (5H, m); 6.30 (1H, s); 5.15, (1H, m); 5.70, (2H, m); 3.15, (2H, d). MS

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TOF 457 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/TFA) rt 13.84 and 14.33 min.

Example 92.

5    3-Amidino-benzoyl-D-phenylglyciny-D-2-naphthylalaniny-D-proline

<sup>1</sup>H nmr (CD<sub>3</sub>CN) δ 8.15 (1H, s); 8.08 (1H, d); 7.95 (1H, d); 7.82 (5H, m); 7.62 (1H, m); 7.22 - 7.5 (7H, m); 5.68 (1H, s); 5.01 (1H, m); 4.25 (1H, m); 3.50 (1H, m);  
10 3.1, (2H, m); 2.22 (1H, m); 2.0 (3H, m); . MS TOF 592 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/TFA) rt 16.04 min.

Example 93.

15    3-Amidino-benzoyl-D-phenylglycine-D-2-naphthylalaniny-L-pipecolic acid

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 7.95 (1H, s); 7.90 (1H, d); 7.85 (1H, d); 7.62 (5H, m); 7.25 (8H, m); 5.60 (1H, s); 5.15 (1H, t); 4.90 (1H, m); 3.60 (1H, m); 3.00 (4H, m); 2.05 (1H, m);  
20 1.45 (2H, m); 1.15 (1H, m); 0.08 (1H, m). MS TOF 606 (M+1<sup>+</sup>). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 14.90 min.

Example 94.

25    3-Amidino-benzoyl-D-phenylglycine-D-2-naphthylalaniny-L-pipecolic acid

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 7.95 (1H, s); 7.90 (1H, d); 7.85 (1H, d); 7.62 (5H, m); 7.25 (8H, m); 5.45 (1H, s); 5.10 (1H, t); 5.00 (1H, m); obscured by solvent 3.60 (1H, m); 3.00 (4H, m);  
30 2.05 (1H, m); 1.45 (2H, m); 1.25 (1H, m); 1.10 (1H, m). MS TOF 606 (M+1<sup>+</sup>). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 14.71 min.

Example 95.

35    3-Amidino-benzoyl-D-naphthylglyciny-D-2-naphthylalaniny-D-proline

<sup>1</sup>H nmr (CD<sub>3</sub>CN) δ 8.13 (1H, s); 8.0 (2H, m); 7.85 (8H, m);

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7.62 , (1H,t); 7.45 (4H, m); 7.05 (2H,m); 6.35 (1H, s);  
5.10 (1H, m); 4.34(1H, m); 3.67 , (2H,m); 3.20, (2H,m);  
2.22 , (1H,m); 2.0 (obscured by solvent) , (3H,m). MS TOF  
642 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1,  
5 water/acetonitrile/TFA) rt 18.14 min.

Prepared by method 3

Example 96.

10 3-Amidino-benzoyl-D-naphthylglyciny1-D-2-naphthylalanine  
amide

<sup>1</sup>H nmr (CD<sub>3</sub>CN) d 8.25 (1H, d); 8.13 (1H, s); 7.95 (H, m);  
7.85 (H, m); 7.74 , (H,m); 7.65 (1H,t); 7.50 (H,m); 7.39  
(2H,d); 6.83 (H,m); 6.22 (1H, s); 4.85(1H, m);  
15 3.25, (2H,m). MS TOF 545 (M+1<sup>+</sup>). Hplc (Jupiter5 C18,  
Gradient 1, water/acetonitrile/TFA) rt 17.32 min.

Prepared by method 4

20 Example 97.

3-Amidino-benzoyl-D-phenylglycine-2-(4-  
methoxyphenyl)ethylamide

<sup>1</sup>H nmr (D4 methanol) 8.20 (2H, m); 8.00 (1H, d);  
7.73(1H, t); 7.40(5H, m); 6.85 (4H, m); 5.60 (1H, m);  
25 3.75 (2H, m); 2.70 (2H, m); 1.85 (3H, s). MS TOF 431  
(M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 10.09 min.

Example 98.

30 3-Amidino-benzoyl-D-phenylglycine-2-(4-  
chlorophenyl)ethylamide

<sup>1</sup>H nmr (D4 methanol) 8.28 (1H, s); 8.21 , (1H,d); 7.97  
(1H, d); 7.73(1H, t); 7.40(5H, m); 7.10 (4H, m); 5.61  
(1H, m); 4.45 (2H, m); 2.78 (2H, m). MS TOF 436 (M+1<sup>+</sup>).  
35 Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA)  
rt 11.26 min.

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**Example 99.****3-Amidino-benzoyl-D-phenylglycine-4-chloro benzylamide**

<sup>1</sup>H nmr (D4 methanol) 8.20 (1H, s); 8.11 , (1H,d); 7.90 (1H, d); 7.65(1H, t); 7.50(5H, m); 7.17 (4H, m); 5.60 (1H, m); 4.31 (2H, m). MS TOF 422 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 10.81 min.

**Example 100.****3-Amidino-benzoyl-D-phenylglycine-4-methoxy benzylamide**

<sup>1</sup>H nmr (D4 methanol) 8.22 (1H, s); 8.15 , (1H,d); 7.93 (1H, d); 7.68 (1H, t); 7.40(5H, m); 6.95 (4H, m); 5.63 (1H, m); 4.30 (2H, m); 3.75 , (3H,s). MS TOF 417 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 9.73 min.

**Example 101.****3-Amidino-benzoyl-D-phenylglycine-3-chloro benzylamide**

<sup>1</sup>H nmr (D4 methanol) 8.32 (1H, s); 8.25 , (1H,d); 7.95 (1H, d); 7.75(1H, t); 7.50(5H, m); 7.20 (4H, m); 5.72 (1H, m); 4.40 (2H, m). MS TOF 422 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 10.78 min.

**Example 102.****3-Amidino-benzoyl-D-phenylglycine-4,4'-dimethyl dibenzylamide**

<sup>1</sup>H nmr (DMSO) 8.30 (1H, s); 8.21 , (1H,d); 7.95 (1H, d); 7.70(1H, t); 7.45(5H, m); 7.10 (10H, m); 6.15 (1H, m); 4.41 (4H, m); 2.30 , (6H,d). MS TOF 505 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 14.39 min.

**Example 103.****3-Amidino-benzoyl-D-phenylglycine-N-methyl-2-phenethylamide**

<sup>1</sup>H nmr (DMSO) 8.25 (1H, s); 8.20 , (1H,d); 7.95 (1H, d); 7.70(1H, t); 7.30 (10H, m); 6.02 (1H, m); 3.78 (1H, m); 3.54 (1H, m); 3.35 , (1H,m); 2.85 , (3H,d); 2.72 , (1H,m).

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MS TOF 415 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 11.65 min.

**Example 104.**

5    **3-Amidino-benzoyl-D-phenylglycine-N-methyl-1-naphthylmethanamide**

<sup>1</sup>H nmr (DMSO) 8.35 (1H, s); 8.30 (1H, d); 8.10 (1H, d); 7.85 (5H, m); 7.60 (4H, m); 7.45 (4H, m); 6.20 (1H, s); 5.15 (2H, m); 2.91 (3H, s). MS TOF 451 (M+1<sup>+</sup>). Hplc  
10 (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 12.53 min.

**Example 105.**

15    **3-Amidino-benzoyl-D-phenylglycine-S-1-naphthylethylamide**  
<sup>1</sup>H nmr (DMSO) 8.25 (1H, s); 8.19 (1H, d); 8.15 (2H, m); 7.95 (2H, m); 7.85 (1H, d); 7.70 (1H, t); 7.55 (5H, m); 7.45 (3H, m); 5.75 (1H, s); 5.70 (1H, m); 1.45 (3H, d). MS TOF 451 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 12.21 min.

20

**Example 106.**

**3-Amidino-benzoyl-D-phenylglycine-1-S-cyclohexylethylamide**

25    <sup>1</sup>H nmr (DMSO) 8.32 (1H, s); 8.20 (1H, d); 7.95 (1H, d); 7.70 (1H, t); 7.50 (2H, m); 7.30 (3H, m); 5.72 (1H, s); 3.55 (1H, m); 0.95-1.80 (11H, m); 0.9 (3H, d). MS TOF 407 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 12.11 min.

30

**Example 107.**

**3-Amidino-benzoyl-D-phenylglycine-+/- (2-methyl)cyclohexylamide**

35    <sup>1</sup>H nmr (DMSO) mixture of isomers (major isomer) 8.29 (1H, s); 8.18 (1H, d); 7.90 (1H, d); 7.70 (1H, t); 7.50 (2H, m); 7.30 (3H, m); 5.70 (1H, s); 0.9-1.8 (8H, m); 0.55 (3H, d). MS TOF 393 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 11.45 min.

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**Example 108.****3-Amidino-benzoyl-D-phenylglycine-exo-2-norbornanamide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.25 (1H, s); 8.17 (1H, d); 7.90 (1H, d);  
7.68 (1H, t); 7.50 (2H, m); 7.30 (3H, m); 5.55 (1H, s);  
5 3.62 (1H, m); 1.05-1.80 (10H, m). MS TOF 391 (M+1<sup>+</sup>).  
Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA)  
rt 11.18 min.

**Example 109.**

10 **3-Amidino-benzoyl-D-phenylglycine-4-  
chlorobenzhydrylamide**

<sup>1</sup>H nmr (DMSO) 8.35 (1H, s); 8.29 (1H, d); 8.03 (1H, d);  
7.79 (1H, t); 7.62 (2H, d); 7.41 (10H, s); 7.18 (2H, d);  
6.23 (1H, s); 6.00 (1H, s). MS TOF 498 (M+1<sup>+</sup>). Hplc  
15 (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt  
13.50 min.

**Example 110.**

20 **3-Amidino-benzoyl-D-phenylglycine-1,1-  
diphenylmethanamide**

<sup>1</sup>H nmr (DMSO) 8.23 (1H, s); 8.17 (1H, d); 7.95 (1H, d);  
7.70 (1H, t); 7.52 (2H, m); 7.35 (11H, s); 7.05 (2H, m);  
6.10 (1H, s); 5.75 (1H, s). MS TOF 464 (M+1<sup>+</sup>). Hplc  
(Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt  
25 12.04 min.

**Example 111.****3-Amidino-benzoyl-D-phenylglycine-4-ethylbenzylamide**

<sup>1</sup>H nmr (DMSO) 8.35 (1H, s); 8.25 (1H, d); 7.95 (1H, d);  
30 7.70 (1H, t); 7.62 (2H, d); 7.41 (5H, m); 7.21 (1H, t);  
6.90 (1H, d); 5.90 (1H, s); 2.60 (2H, q); 1.20 (3H, t).  
MS TOF 401 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 12.03 min.

35



**Example 112.****3-Amidino-benzoyl-D-phenylglycine-2-tertiary butyl anilide**

<sup>1</sup>H nmr (DMSO) 8.21 (1H, s); 8.12 (1H, d); 7.85 (1H, d);  
5 7.60 (1H, t); 7.52 (2H, d); 7.25 (4H, m); 7.10 (2H, m);  
6.90 (1H, m); 5.90 (1H, s); 1.00 (9H, s). MS TOF 429  
(M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 12.05 min.

10 **Example 113.****3-Amidino-benzoyl-D-phenylglycine-3-(1-hydroxyethyl) anilide**

<sup>1</sup>H nmr (DMSO) 8.45 (1H, s); 8.35 (1H, d); 8.08 (1H, d);  
7.88 (1H, t); 7.75 (3H, m); 7.55 (4H, m); 7.38 (1H, t);  
15 7.20 (1H, d); 6.02 (1H, s); 4.80 (1H, m); 1.45 (3H, d).  
MS TOF 417 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 9.12 min.

**Example 114.**20 **3-Amidino-benzoyl-D-phenylglycine-4-(4'-acetylphenyl)piperazinamide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.12 (1H, s); 8.05 (1H, d); 7.87 (1H, d);  
7.80 (2H, d); 7.65 (1H, t); 7.41 (5H, m); 6.80 (2H, d);  
6.05 (1H, s); 3.80 (1H, m); 3.65 (2H, m); 3.45 (2H, m);  
25 3.35 (1H, m); 3.20 (1H, m); 2.75 (1H, m); 2.45 (3H, s). MS  
TOF 485 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1,  
water/acetonitrile/TFA) rt 12.96 min.

**Example 115.**30 **3-Amidino-benzoyl-D-phenylglycine-4-(4'-methoxyphenyl)piperazinamide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.15 (1H, s); 8.08 (1H, d); 7.91 (1H, d);  
7.68 (2H, d); 7.45 (1H, t); 7.41 (5H, m); 6.99 (2H, d);  
6.12 (1H, s); 3.95 (3H, m); 3.78 (3H, s); 3.75 (2H, m);  
35 3.55 (2H, m); 3.45 (1H, m); 2.92 (1H, m). MS TOF 473  
(M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1,  
water/acetonitrile/TFA) rt 10.34 min.

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**Example 116.****3-Amidino-benzoyl-D-phenylglycine-4-(4'-chlorophenyl)piperazinamide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.05 (1H, s); 8.00 (1H, d); 7.87 (1H, d);  
5 7.55 (1H, t); 7.31 (5H, m); 7.08 (2H, d); 6.75 (2H, d);  
5.95 (1H, s); 3.70 (1H, m); 3.55 (2H, m); 3.45 (1H, m);  
3.12 (1H, m); 3.00 (1H, m); 2.85 (1H, m); 2.35 (1H, m). MS  
TOF 477 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1,  
water/acetonitrile/TFA) rt 17.80 min.

10

**Example 117.****3-Amidino-benzoyl-D-phenylglycine-4-phenylpiperazinamide**

<sup>1</sup>H nmr (DMSO) 8.28 (1H, s); 8.21 (1H, d); 7.94 (1H, d);  
7.71 (1H, t); 7.52 (2H, d); 7.35 (3H, m); 7.21 (2H, m);  
15 6.89 (2H, d); 6.80 (1H, t); 6.15 (1H, s); 3.75 (3H, m);  
3.55 (1H, m); 3.15 (2H, m); 3.00 (1H, m); 2.78 (1H, m). MS  
TOF 442 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 9.99 min.

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**Example 118.****3-Amidino-benzoyl-D-phenylglycine-4-toluenesulphonyl  
piperazinamide**

<sup>1</sup>H nmr (DMSO) 8.21 (1H, s); 8.11 (1H, d); 7.91 (1H, d);  
7.69 (1H, t); 7.50 (4H, m); 7.35 (2H, m); 7.31 (3H, m);  
25 6.05 (1H, s); 3.60 (3H, m); 3.45 (2H, m); 2.90 (1H, m);  
2.78 (1H, m); 2.70 (1H, m); 2.45 (3H, s); 2.25 (1H, m). MS  
TOF 521 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 10.76 min.

30

**Example 119.****3-Amidino-benzoyl-D-phenylglycine-4-benzoyl  
piperazinamide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 7.95 (1H, s); 7.88 (1H, d); 7.70 (1H, d);  
7.45 (1H, t); 7.20 (10H, m); 5.85 (1H, s, broad); 2.50-  
35 3.60 (8H, m, broad signals). MS TOF 470 (M+1<sup>+</sup>). Hplc  
(Jupiter5 C18, Gradient 1, water/acetonitrile/TFA) rt  
10.78 min.

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**Example 120.****3-Amidino-benzoyl-D-phenylglycine piperidinamide**

<sup>1</sup>H nmr (DMSO) 8.20 (1H, s); 8.10 (1H, d); 7.86 (1H, d);  
7.65 (1H, t); 7.45 (5H, m); 6.02 (1H, s); 3.70 (1H, m);  
5 3.40 (3H, m); 1.53 (3H, m); 1.41 (2H, m); 0.93 (1H, m).  
MS TOF 365 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 9.29 min.

**Example 121.**

10 **3-Amidino-benzoyl-D-phenylglycine-(1S, 2S, 3S, 5R)-  
isopinocampyl amide**

<sup>1</sup>H nmr (DMSO) 8.30 (1H, s); 8.21 (1H, d); 7.98 (1H, d);  
7.71 (1H, t); 7.51 (2H, m); 7.35 (3H, m); 5.74 (1H, s);  
4.05 (1H, m); 2.35 (2H, m); 1.90 (1H, m); 1.72 (2H, m);  
15 1.60 (1H, m); 1.21 (3H, s); 1.01 (1H, d); 0.97 (3H, s); 0.83  
(3H, d). MS TOF 433 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient  
3, water/acetonitrile/TFA) rt 13.32 min.

**Example 122.**

20 **3-Amidino-benzoyl-D-phenylglycine-3-phenylanilide**

<sup>1</sup>H nmr (DMSO) 8.31 (1H, s); 8.24 (1H, d); 7.95 (2H, m);  
7.53 (1H, t); 7.60 (5H, m); 7.4 (8H, m); 5.85 (1H, s).  
MS TOF 449 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 12.42 min.

25

**Example 123.**

**3-Amidino-benzoyl-D-phenylglycine-3-(O-benzoyl  
hydroxymethyl) anilide**

<sup>1</sup>H nmr (DMSO) 8.40 (1H, d); 8.33 (1H, s); 8.31 (1H, d);  
30 7.97 (2H, d); 7.75 (1H, t); 7.64 (1H, m); 7.56 (2H, d); 7.40  
(8H, m); 7.21 (1H, t); 5.75 (1H, s); 5.20 (2H, s). MS TOF  
507 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 13.53 min.

35

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**Example 124.****3-Amidino-benzoyl-D-phenylglycine-4-(4-chlorobenzoyl)  
piperidinamide**

<sup>1</sup>H nmr (DMSO) Mixture of rotomers 8.08 (2H, m); 7.80 (3H, m); 7.55 (1H, t); 7.45 (2H, m); 7.25 (5H, m); 5.92 (1H, s); 4.25 (1H, m); 3.80 (1H, m); 3.55 (1H, m); 2.90 (1H, m); 2.68 (1H, m); 1.60 (1H, m); 1.55 (1H, m); 1.30 (1H, m); 0.83 (1H, m). MS TOF 504 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 12.21 min.

**Example 125.****3-Amidino-benzoyl-D-phenylglycine-4-(4-fluorobenzoyl)  
piperidinamide**

<sup>1</sup>H nmr (DMSO) Mixture of rotomers 8.40 (2H, m); 8.22 (2H, m); 8.10 (1H, d); 7.88 (1H, t); 7.64 (2H, m); 7.54 (5H, m); 6.30 (1H, s); 4.65 (1H, m); 4.20 (1H, m); 3.82 (1H, m); 3.10 (2H, m); 1.98 (1H, m); 1.70 (1H, m); 1.40 (1H, m); 0.83 (1H, m). MS TOF 487 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 11.37 min.

**Example 126.****3-Amidino-benzoyl-D-phenylglycine-4-benzyl  
piperidinamide**

<sup>1</sup>H nmr (DMSO) Mixture of rotomers 7.98 (2H, m); 7.68 (1H, m); 7.45 (1H, m); 6.60-7.25 (10H, m); 5.86 (1H, s); 4.15 (1H, m); 3.68 (1H, m); 2.63 (1H, m); 2.45 (1H, m); 2.21 (3H, m); 1.50 (1H, m); 1.33 (1H, m); 1.03 (1H, m); 0.45 (1H, m). MS TOF 455 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 13.18 min.

**Example 127.****3-Amidino-benzoyl-D-phenylglycine-D-2-naphthylalanine  
pyrrolidinamide**

<sup>1</sup>H nmr (DMSO) 8.19 (1H, s); 8.11 (1H, d); 7.92 (1H, d); 7.75 (5H, m); 7.41 (5H, m); 7.30 (3H, m); 5.81 (1H, s); 4.80 (1H, m); 3.20 (4H, m); 3.00 (2H, m); 1.55 (4H, m). MS TOF 548 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,

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water/acetonitrile/TFA) rt 11.13 min.

**Example 128.**

**3-Amidino-benzoyl-DL-1-naphthylglyciny-D-2-naphthylalanine pyrrolidinamide**

<sup>1</sup>H nmr (DMSO) mixture of isomers 8.25 (1H, s); 8.20 (1H, d); 8.15 (2H, m); 8.05 (1H, d); 6.90-8.00 complex multiplet (13H, m); 6.50 (1H, m); 4.90 (1H, m); 2.90-3.50 broad, partially covered by solvent (H, m); 1.70 (4H, m). MS TOF 598 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 12.18 and 12.37 min.

**Example 129.**

**3-Amidino-benzoyl-DL-1-naphthylglycine-4-methylbenzylamine**

<sup>1</sup>H nmr (DMSO) mixture of isomers 8.25 (1H, s); 8.20 (1H, d); 8.10 (1H, m); 7.90 (3H, m); 7.70 (1H, t); 7.55 (4H, m); 7.06 (4H, m); 6.45 (1H, s); 4.30 (2H, m); 2.25 (3H, s). MS TOF 451 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 12.30 min.

**Example 130.**

**3-Amidino-benzoyl-DL-1-naphthylglycine-4-benzoyl piperidinamide**

<sup>1</sup>H nmr (DMSO) mixture of isomers 8.15 (2H, m); 8.00 (1H, d); 7.85 (5H, m); 7.45 (8H, m); 6.70 (1H, s); 4.50 (1H, m); 3.15 (1H, m); 2.80 (3H, m); 1.60 (2H, m); 1.42 (1H, m); 0.45 (1H, m). MS TOF 519 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 12.67 min.

**Example 131.**

**3-Amidino-benzoyl-DL-1-naphthylglycine-3-(1-hydroxyethyl)anilide**

<sup>1</sup>H nmr ( ) mixture of isomers 8.10 (1H, s); 8.00 (1H, d); 7.80 (3H, m); 7.50 (7H, m); 7.30 (1H, m); 7.20 (1H, m); 7.00 (1H, m); 6.45 (1H, s); 4.65 (1H, m); 1.20 (3H, s). MS TOF 467 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,

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water/acetonitrile/TFA) rt 10.67 min.

**Example 132.**

**3-Amidino-benzoyl-D-phenylglycine morpholinamide**

5 <sup>1</sup>H nmr (DMSO) 8.25 (1H, s); 8.18 (1H, d); 7.91 (1H, d);  
7.68 (1H, t); 7.40 (5H, m); 6.09 (1H, s); 5.45 (4H, m);  
3.63 (4H, m). MS TOF 367 (M+1<sup>+</sup>). Hplc (Jupiter5 C18,  
Gradient 3, water/acetonitrile/TFA) rt 5.53 min.

10 **Example 133.**

**3-Amidino-benzoyl-R,S-3-amino-3-phenylpropionic-1-adamantylamide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.03 (1H, s); 7.97 (1H, d); 7.75 (1H, d);  
7.55 (1H, t); 7.20 (5H, m); 5.25 (1H, t); 2.50-1.70 (15H,  
15 m). MS TOF 446 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 11.56 min.

**Example 134.**

**3-Amidino-benzoyl-DL-1-naphthylglycine-(Z,E)-(2-methyl)cyclohexylamide**

20 Mixture of isomers. MS TOF 443 (M+1<sup>+</sup>). Hplc (Jupiter5  
C18, Gradient 3, water/acetonitrile/TFA) rt 12.57 and  
12.75 min.

25 **Prepared by Method 5**

**Example 135.**

**3-Amidino-benzoyl-D-phenylglycine cyclopropylmethyleamide**

MS TOF 351 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
30 water/acetonitrile/TFA) rt 8.57 min.

**Example 136.**

**3-Amidino-benzoyl-D-phenylglycine-2-indanylamide**

MS TOF 413 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
35 water/acetonitrile/TFA) rt 11.13 min.

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**Example 137.****3-Amidino-benzoyl-D-phenylglycine-3-methylbutylamide**MS TOF 367 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 10.81 min.

5

**Example 138.****3-Amidino-benzoyl-D-phenylglycine-4-fluorobenzylamide**MS TOF 405 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 10.41 min.

10

**Example 139.****3-Amidino-benzoyl-D-phenylglycine trans-2-phenylcyclopropylamide**MS TOF 413 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 11.25 min.

15

**Prepared by Method 6****Example 140.****20 3-Amidino-benzoyl-D-phenylglycine-2-(4-N-acetyl piperidinyl)ethyl ester**

<sup>1</sup>H nmr (DMSO) 8.13 (1H, s); 8.07 (1H, d); 7.92 (1H, d); 7.69 (2H, d); 7.45 (5H, m); 5.63 (1H, s); 4.25 (2H, m); 3.65 (1H, m); 3.45 (3H, m); 2.80 (1H, m); 3.35 (1H, m); 2.00 (3H, s); 1.50 (3H, m); 1.30 (1H, m); 0.95 (1H, m). MS TOF 451 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt 19.00 min.

25

**Method 7**

30

By solution phase strategy: Typically a Boc-amino alcohol with TEA is treated with an acid chloride to give an ester. Removal of the Boc protecting group with TFA followed by further extension of the compounds as in Method 4.

35

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**Example 141.**

{R}-[N-(3-amidino)benzoyl]amino}-2-phenylethyl-4-(methoxy)benzoate.

5 To Boc-(D)-phenylglycinol (237mg, 1mmol, 1eq) and TEA(153 $\mu$ l, 1.1mmol, 1eq) dry DCM (5ml) was added p-anisoyl chloride (188mg, 1.1mmol, 1.1eq). The reaction was stirred overnight. Ethylacetate (30ml) was added and the organic layer was washed successively with 2 x  
10 10ml of 10%HCl, NaHCO<sub>3</sub> solution, finally brine. Dried over magnesium sulphate, filtered and evaporated. to give the crude product (410mg). After treatment with TFA to remove the Boc group the compound were extended as in Method 4.

15

<sup>1</sup>H nmr (DMSO) 8.25 (1H, s); 8.21 (1H, d); 7.95 (1H, d); 7.85 (2H,d); 7.75 (1H, t); 7.45 (5H, m); 7.05 (2H,d); 5.55 (1H, q); 4.55 (2H, d); 3.85 (3H, s). MS TOF 418 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 11.01 min.

20

By analogous methods the following were prepared

**Example 142.**

25 R-2-[N-(3-amidino)benzoyl]amino}-2-phenylethyl-4-(methyl)benzoate

<sup>1</sup>H nmr (DMSO) 8.25 (1H, s); 8.21 (1H, d); 7.95 (1H, d); 7.80 (2H,d); 7.75 (1H, t); 7.50 (2H,d); 7.34 (5H, m); 5.55 (1H, q); 4.55 (2H, d); 3.35 (3H, s). MS TOF 402 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 11.87 min.

30

**Example 143.**

35 R-2-[N-(3-amidino)benzoyl]amino}-2-phenylethyl-4-(trifluoromethoxy)benzoate

<sup>1</sup>H nmr (DMSO) 8.45 (1H, s); 8.40 (1H, d); 8.25 (2H,d); 8.15 (1H, d); 7.95 (1H, t); 7.65 (7H, m); 5.78 (1H, q);



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4.84 (2H, d). MS TOF 472 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 12.85 min.

#### Method 8

5

By solution phase strategy: Typically a Boc-amino alcohol and a carboxylic acid are treated with (4-dimethylamino) phenyldiphenyl phosphine and diethylazodicarboxylate to give an ester. Removal of the Boc protecting group with TFA followed by further extension of the compounds as in Method 4.

#### Example 144.

{R}-{[N-(3-amidino)benzoyl]amino}-2-phenylethyl-4-(acetimido)benzoate

A dry solution of diethylazodicarboxylate (158 $\mu$ l, 1mmol, 1eq) in THF (5ml), was added dropwise to an anhydrous solution of (4-dimethylamino)phenyldiphenyl phosphine (306mg, 1eq, 1mmol), Boc-(D)-phenylglycinol (356mg, 1.5eq, 1mmol) and 4-acetimidobenzoic acid (179mg, 1mmol, 1eq) in THF (5ml) at -78°C. The reaction allowed to warm to room temperature and stirred overnight. Reaction monitored by t.l.c, SiO<sub>2</sub> / 75% ethylacetate in hexane indicating formation of product. R<sub>f</sub> = 0.5. The solution was evaporated to remove THF and taken up into ethyl acetate (30ml) washed with 10% HCl solution, then brine. The solution was dried, filtered and evaporated to dryness. Crude yield ~650mg. Purification by column chromatography gave a yield of product of 200mg, (50%). After treatment with TFA to remove the Boc group the compound were extended as in Method 4.

<sup>1</sup>H nmr (DMSO) 8.48 (1H, s); 8.54 (1H, d); 8.15 (1H, d); 8.05 (2H, d); 7.95 (3H, m); 7.75 (2H, m); 7.55 (3H, m); 5.70 (1H, m); 4.70 (2H, m); 2.27 (3H, s). MS TOF 445 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,

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water/acetonitrile/TFA) rt 9.38 min.

**Example 145.**

**R-2-{[N-(3-amidino)benzoyl]amino}-2-phenylethyl-4-(methylsulphonyl)benzoate**

<sup>1</sup>H nmr (DMSO) 8.25 (1H, s); 8.21 (1H, d); 8.10 (4H, q); 7.95 (1H, d); 7.75 (1H, t); 7.45 (5H, m); 5.60 (1H, q); 4.65 (2H, d); 2.08 (3H, s). MS TOF 466 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 9.75 min.

**Example 146.**

**R-2-{[N-(3-amidino)benzoyl]amino}-2-phenylethyl-4-acetylbenzoate**

<sup>1</sup>H nmr (DMSO) 8.03 (1H, s); 8.00 (1H, d); 7.83 (4H, m); 7.73 (1H, d); 7.53 (1H, t); 7.25 (5H, m); 5.38 (1H, q); 4.41 (2H, d); 2.42 (3H, s). MS TOF 430 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 10.46 min.

**Method 9**

By solution phase strategy: Typically an activated Boc-amino acid was converted to the amide with ammonia and then to the thioamide using Lawessons reagent. Reaction of the thioamide with an acyl bromide to give a Boc protected thiazole. Deprotection of the thiazole with TFA allows further extension of the compound as in Method 4.

**Example 147.**

**2-[(N-3-amidinobenzamido)-1-phenyl]methyl-4-phenylthiazole**

To a solution of Boc-D-phenylglycine (875mg 3.5mmole) in a 1:1 mixture of DMF and DCM (40ml) was added 1-hydroxybenzotriazole (520mg 1.1equiv.) and DIPCI (602ml

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1.1equiv.) and the mixture stirred for 30min at rt. Ammonia gas was bubbled in and the mixture left overnight before diluting with ethyl acetate and washing with 10% hydrochloric acid and sat. sodium bicarbonate.

5 The organic solution was dried (magnesium sulphate) and evaporated. Flash chromatography (silica gel DCM/ethylacetate 0-50%) gave the amide 770mg 86%. To a solution of Boc-D-phenylglycine amide (740mg 2.96mmole) in THF 25ml was added Lawesson's reagent

10 (1.2g) and the mixture stirred overnight. The solvent was evaporated off under reduced pressure and the residue purified by flash chromatography (silica gel - hexane/ethyl acetate 10 to 30%) to give the thioamide 671mg.

15 The thioamide (650mg) was dissolved in acetone (20ml) and phenacyl bromide (486mg 1 equiv) stirred for 30min and then diluted with chloroform/aqueous sod. bicarbonate (20ml each), separated, dried (magnesium sulphate) and evaporated. The residue was dissolved in

20 DCM (20ml) and treated with pyridine (350ml) and trifluoroacetic anhydride (360ml). After 150min the solvent was removed under reduced pressure and the residues redissolved in DCM and washed with sat. sod. bicarbonate. Purification by flash chromatography

25 (silica gel - hexane/ethyl acetate 10 to 30%) gave the thiazole intermediate 687mg. Deprotection of the amine was carried out using 50% TFA in DCM (30min), evaporation of solvent under reduced pressure, dissolving in DCM, washing with sat sod.

30 bicarbonate, drying (magnesium sulphate) and evaporation. Coupling of the thiazole intermediate to 3-amidino benzoic acid was carried out using the standard method Method 4 to give 2-[(N-3-amidinobenzamido)-1-

35 phenyl]methyl-4-phenylthiazole TFA salt.

<sup>1</sup>HNMR (d<sub>6</sub>DMSO) 9.9 (1H, d); 9.48 (1H, s); 9.09 (1H, s);

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8.40 (1H,s); 8.37 (1H, d); 8.16 (1H, s); 7.98 (3H, m);  
7.80 (2H,t); 7.63 (2H, d); 7.5 (6H, m); 6.80 (1H, d). MS  
TOF 413 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 11.8 min.

5

**Method 10**

By solution phase: Typically a compound prepared as  
Method 4 is further reacted with ethyl chloroformate to  
10 give the 3-Ethoxycarbonyl amidino compound

**Example 148.**

**3-(Ethoxycarbonyl)-amidinobenzoyl-D-phenylglycine-4-  
methylbenzyl amide.**

15

To a solution of 3-Amidinobenzoyl-(D)-phenylglycine-4-  
methylbenzyl amide (125mg, 0.31mmole) in DCM (20ml) was  
added DIPEA (163ml 0.94mmole) and then over 2min. ethyl  
chloroformate 33ml 0.34mmole). The reaction was stirred  
20 overnight and the solvent removed under reduced  
pressure. The residue was dissolved in ethylacetate and  
washed with water (x3), dried (magnesium sulphate) and  
evaporated to dryness. Flash chromatography (silica gel,  
DCM/Ethylacetate 0-100%) gave the title compound 98mg

25

<sup>1</sup>HNMR (d<sub>6</sub>DMSO) 9.1 (2H, bs); 8.93 (1H,d); 8.82 (1H, t);  
8.46 (1H, s); 8.08 (2H, t); 7.53 (3H, m); 7.36 (3H, m);  
7.08 (4H, s); 5.76 (1H,d); 4.23 (2H, d); 4.07 (2H, q);  
2.25 (3H, s); 1.22 (3H,t). MS TOF 473 (M+1<sup>+</sup>). Hplc  
30 (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt  
11.06 min.

By an analogous method

35

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**Example 149****3-(Ethoxycarbonylamidino)-D-phenylglycine-4-(4-chlorophenyl)-piperazinamide**

<sup>1</sup>HNMR (d<sub>6</sub>DMSO) 9.01 (3H, bs+s); 8.40 (1H, s); 8.08 (2H, m); 7.52 (3H, m); 7.40 (3H, m); 7.25 (2H, d); 6.88 (2H, d); 6.15 (1H, d); 4.05 (2H, d); 3.70 (4H, m); 3.01 (4H, m); 1.20 (3H, t). MS TOF 549 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 12.48 min.

**10 Method 11**

By solution phase strategy: Typically 3-cyanobenzoic acid is activated with carbonyl-diimidazole and reacted with R-phenylglycinol to give 3-cyanobenzoylphenylglycinol. Reaction with a trichloroacetimidate and BF<sub>3</sub> etherate, (4), gives an ether. Reaction of the cyano group with HCl in ethanol followed by ammonia in ethanol gives the amidino compound.

**20 Example 150.****3-Amidinobenzoyl-2-(4-methylbenzyloxy)-1-phenylethylamide**

3-Cyanobenzoic acid was condensed with (R)-phenylglycinol using N,N'-carbonyl-diimidazole as activator to give 3-cyanobenzoylphenylglycinol. <sup>1</sup>HNMR (CDCl<sub>3</sub>) 8.05 (2H, d); 7.55 (1H, t); 7.4 (8H, m); 5.17 (2H, bs); 4.50 (2H, bs).

3-Cyanobenzoylphenylglycinol in 1:1 DCM: cyclohexanol (50ml) was treated with 4-methylbenzyl trichloroacetimidate and BF<sub>3</sub> etherate and the crude product purified by flash chromatography to give 3-cyanobenzoyl-2-(4-methylbenzyloxy)-1-phenylethylamide which was then taken up in ethanol and saturated with HCl gas and left overnight at room temperature. The solvent and excess HCl gas were removed under reduced

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pressure and the residue redissolved in ethanol and the solution saturated with ammonia gas and left overnight. The solvent and excess ammonia were removed under reduced pressure and the residue treated with aqueous acetonitrile and trifluoroacetic acid. Preparative hplc gave the title compound approx 80% pure by hplc.

<sup>1</sup>HNMR (d<sub>6</sub>DMSO) 9.1 (5H, bs+d); 8.22 (2H, s+d); 7.90 (1H, d); 7.74 (1H, t); 7.35 (5H, m); 7.10 (4H, Abq); 5.35 (1H, q); 4.50 (2H, s); 3.70 (2H, m); 2.25 (3H, s). MS TOF 389 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 11.79 min.

#### Miscellaneous Examples

##### Example 151.

**N-(3-Amidinobenzoyl)-2-amino-2(R)-phenylethoxycarbonyl-4-methylaniline**

To a solution of Boc-(R)-phenylglycinol (500mg 3.65mmole) in DMF (20ml) was added 4-methylphenylisocyanate (500ml 4.0mmole) and the mixture warmed to 50°C before standing at room temperature overnight. Dilution with ethyl acetate and washing with sat. sodium bicarbonate gave after drying (magnesium sulphate) and evaporation of solvent the crude intermediate which was purified by flash chromatography (silica gel, EtOAc 0-20% in DCM)

Deprotection of the amine was carried out using 50% TFA in DCM (30min), evaporation of solvent under reduced pressure, dissolving in DCM, washing with sat sod. bicarbonate, drying (magnesium sulphate) and evaporation.

Coupling of the thiazole intermediate to m-benzamidine was carried out using the standard method 4 to give N-(3-Amidinobenzoyl)-2-amino-2(R)-phenylethoxycarbonyl-4-methylaniline.

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<sup>1</sup>H NMR (d<sub>6</sub>DMSO) 8.2 (2H, m); 7.90 (1H, d); 7.72 (1H, t); 7.44 (2H, d); 7.35 (5H, m); 7.08 (2H, d); 5.35 (1H, m); 4.33 (2H, m); 2.25 (3H, s). MS TOF 417 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 11.54 min.

**Example 152.****3-Amidinobenzyl-D-phenylglycine-cis/trans-4-aminomethyl cyclohexylmethanamide**

10

Bis-1,4-aminomethylcyclohexane 2-chlorotrityl resin (0.2 mmol) (as prepared by method 1) was treated with Fmoc-D-Phenylglycine (0.5 mmol, 187mg), DMF (2.5ml), TBTU in DMF (1.25ml of a 450mM solution) and DIPEA in DMF (1.25ml of a 900 mM solution). The mixture was agitated with nitrogen for 2 hours. Deprotection and washing as in method 1. The resin was then treated with 3-cyanobenzaldehyde (0.5mmol, 66mg) in DMF agitated overnight. The resin was washed with DMF then with Dry THF to remove all DMF. Sodium cyanoborohydride (1mmol, 63mg) in THF and acetic acid (100μl) was then added to the resin and agitated for 4 hours. The resin was then washed with THF, the DMF and finally DCM. The resin was then treated with TFA containing 5% TES for 30 mins. The resin was then filtered off and the TFA evaporated then held under high vacuum to dry the product. The product was then taken up into saturated hydrogen chloride gas in ethanol (20ml.) overnight. The ethanol was then evaporated and the product treated with saturated ammonia gas in ethanol (20ml.) overnight. The ethanol was then evaporated and the product purified by preparative hplc.

<sup>1</sup>H nmr (CD<sub>3</sub>CN) Mixture of cyclohexyl cis and trans isomers 7.85 (2H, m); 7.79 (1H, d); 7.66 (1H, t); 7.50 (5H, m); 4.97 (1H, s); 4.22 (2H, s); 3.00 (2H, m); 2.78 (2H, m); 1.48 (7H, m); 0.86 (3H, m) MS TOF 407 (M+1<sup>+</sup>).

35

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Hplc (Jupiter5 C18, Gradient 2, water/acetonitrile/TFA)  
rt 9.78 min.

**Example 153.****5 4-Methylphenylacetyl-L-phenylglycine-3-amidino anilide**

Boc-L-phenylglycine (0.6mmol, 150mg), HATU (0.6mmol, 230mg) and DIPEA (1.2mmol., 220 $\mu$ l) were dissolved in DMF (5ml) and stirred for 15mins. 3-Aminobenzonitrile (0.6mmol.,  
10 70mg) was added and the mixture stirred overnight. The DMF was then evaporated and the mixture taken into ethylacetate (50ml) and washed with water, sodium carbonate solution, water 10% hydrochloric acid and water. The ethylacetate was evaporated to give the product Boc-L-phenylglycine-3-cyano anilide. The product was treated  
15 with TFA (50ml) for 30mins. The TFA was evaporated to dryness. m-Tolylacetic acid (0.7mmol., 100mg), HATU (0.7mmol., 250mg) and DIPEA (1.4mmol., 250 $\mu$ l) were dissolved in DMF (5ml) and stirred for 15mins. This was then added to the above product with DIPEA (0.6mmol.,  
20 110 $\mu$ l) and stirred overnight. The DMF was evaporated and the mixture taken into ethylacetate (50ml) and washed as above. The ethyl acetate was then evaporated and the product treated with hydrogen chloride gas in ethanol and ammonia gas in ethanol as above. The product was purified  
25 by preparative hplc.

<sup>1</sup>HNMR (d, MeOH) 8.23 (1H, s); 7.90 (1H, d); 7.72 (7H, m);  
7.44 (1H, t); 7.29 (3H, m); 5.75 (1H, m); 3.80 (2H, s);  
30 2.50 (3H, s). MS TOF 401 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/TFA) rt 14.59 min.

**Compounds synthesised by Method 4**

35



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**Example 154.****3-Amidino-benzoyl-D-phenylglycine-4-(4'-nitrophenyl)  
piperazinamide**

5 <sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.20 (1H, s); 8.05 (3H, m); 7.85 (1H, d);  
7.70 (1H, t); 7.40 (5H, m); 6.82 (2H, d); 6.05 (1H, s);  
3.75 (1H, m); 3.65 (2H, m); 3.45 (3H, m); 3.30 (1H, m); 2.85  
(1H, m). MS TOF 486 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient  
1, water/acetonitrile/TFA) rt 11.65 min.

10

**Example 155.****3-Amidino-benzoyl-D-phenylglycine-4-(4'-aminophenyl)  
piperazinamide**

15 <sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.00 (1H, s); 7.85 (1H, d); 7.65 (1H, d);  
7.45 (1H, t); 7.20 (5H, m); 6.95 (2H, d); 6.70 (2H, d);  
5.85 (1H, s); 3.55 (1H, m); 3.30 (1H, m); 2.95 (3H, m);  
2.80 (1H, m); 2.40 (1H, m). MS TOF 458 (M+1<sup>+</sup>). Hplc  
(Jupiter5 C18, Gradient 2, water/acetonitrile/TFA) rt  
20 12.57 min.

**Example 156.****3-Amidino-benzoyl-D-phenylglycine-4-(4'-fluorophenyl)  
piperazinamide**

25

<sup>1</sup>H nmr (CD<sub>3</sub>OD) 8.25 (1H, s); 8.20 (1H, d); 7.95 (1H, d);  
7.75 (1H, t); 7.45 (5H, m); 7.10 (4H, m); 6.20 (1H, s);  
3.90 (3H, m); 3.70 (1H, m); 3.35 (2H, m); 3.15 (1H, m); 2.65  
(1H, m). MS TOF 460 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient  
30 1, water/acetonitrile/TFA) rt 14.88 min.

**Example 157.****3-Amidino-benzoyl-D-phenylglycine-4-(4'-pyridyl)  
piperazinamide**

35

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.05 (1H, s); 7.95 (1H, d); 7.90 (2H, d);  
7.80 (1H, d); 7.55 (1H, t); 7.30 (5H, m); 6.80 (2H, d);

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6.00 (1H, s); 3.70 (3H,m); 3.50 (3H,m); 3.40 (1H,m);  
3.15 (1H,m). MS TOF 444 (M+1<sup>+</sup>). Hplc (Jupiter5 C18,  
Gradient 2, water/acetonitrile/TFA) rt 11.54 min.

5     **Example 158.**

**3-Amidino-benzoyl-D-phenylglycine-4-(2'-pyridyl)  
piperazinamide**

<sup>1</sup>H nmr (CD<sub>3</sub>OD) 8.30 (1H, s); 8.20 (1H, d); 8.10 (1H, m);  
10     7.95 (2H,m); 7.75 (1H, t); 7.50 (5H, m); 7.30 (2H,d);  
7.15 (1H, t); 6.20 (1H, s); 3.95 (3H,m); 3.75 (4H,m);  
3.40 (1H,m). MS TOF 444 (M+1<sup>+</sup>). Hplc (Jupiter5 C18,  
Gradient 2, water/acetonitrile/TFA) rt 11.67 min.

15     **Example 159.**

**3-Amidino-benzoyl-D-phenylglycine-4-benzylpiperazinamide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.15 (1H, s); 8.05 (1H, d); 7.90 (1H, d);  
7.65 (1H, t); 7.40 (10H, m); 6.05 (1H, s); 4.20 (2H,s);  
20     3.20 (8H,v. broad m). MS TOF 457 (M+1<sup>+</sup>). Hplc (Jupiter5  
C18, Gradient 1, water/acetonitrile/TFA) rt 9.52 min.

**Example 160.**

**3-Amidino-benzoyl-D-phenylglycine-4-(3-chlorophenyl)  
25     piperazinamide**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.17 (1H, s); 8.07 (1H, d); 7.90 (1H, d);  
7.65 (1H, t); 7.42 (5H, m); 7.15 (1H, m); 6.80 (3H,m);  
6.08 (1H, s); 3.85 (1H,m); 3.55 (3H,m); 3.25 (1H,m);  
30     3.10 (1H,m); 2.95 (1H,m); 2.40 (1H,m). MS TOF 477  
(M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1,  
water/acetonitrile/TFA) rt 15.39 min.

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**Example 161.****3-Amidino-benzoyl-D-phenylglycine-4-(2-chlorophenyl)  
piperazinamide**

5 <sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.15 (1H, s); 8.07 (1H, d); 7.90 (1H, d);  
7.65 (1H, t); 7.42 (5H, m); 7.20 (1H, m); 6.95 (3H, m);  
6.05 (1H, s); 3.80 (1H, m); 3.65 (2H, m); 3.45 (1H, m); 3.  
00 (1H, m); 2.85 (2H, m); 2.40 (1H, m). MS TOF 477 (M+1<sup>+</sup>).  
Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/TFA)  
10 rt 15.02 min.

**Example 162.****3-Amidino-benzoyl-D-phenylglycine-tetrahydroisoquinolin-  
2-amide**

15 <sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.05 (1H, s); 8.00 (1H, d); 7.70 (1H, d);  
7.45 (1H, t); 7.25 (2H, m); 7.15 (3H, m); 6.90 (4H, m);  
6.00 (1H, s); 4.45 (2H, m); 3.45 (2H, m); 2.55 (2H, m).  
MS TOF 413 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
20 water/acetonitrile/TFA) rt 10.92min.

**Example 163.****3-Amidino-benzoyl-D-phenylglycinyln-N-benzyl glycine  
ethyl ester**

25 <sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.15 (1H, s); 8.10 (1H, d); 7.90 (1H, d);  
7.70 (1H, t); 7.35 (10H, m); 6.10 (1H, s); 4.65 (2H, m);  
4.45 (2H, m); 4.05 (2H, m); 1.15 (3H, m). MS TOF 473  
(M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
30 water/acetonitrile/TFA) rt 11.32 min.

**Example 164.****3-Amidino-benzoyl-D-phenylglycinyln-1,2,3,4-DL-  
tetrahydroisoquinoline-3-dimethylamide**

35 <sup>1</sup>H nmr (CD<sub>3</sub>OD) mixture of isomers, major reported. 8.25  
(1H, s); 8.20 (1H, d); 7.95 (1H, d); 7.75 (1H, t); 7.70 -

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7.00 (9H, m); 6.35 (1H, s); 5.20 (1H, m); 4.65 (2H, m);  
3.15 (2H, m, obscured by solvent); 3.00 (6H, d). MS TOF  
484 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1,  
water/acetonitrile/TFA) rt 13.81 min.

5

#### Compounds synthesised by Method 7

##### Example 165.

R-2-{[N-(3-amidino)benzoyl]amino}-2-phenylethyl-4-  
10 (methyl)benzoate

<sup>1</sup>H nmr (DMSO) 8.25 (1H, s); 8.21 (1H, d); 7.95 (1H, d);  
7.80 (2H, d); 7.75 (1H, t); 7.50 (2H, d); 7.34 (5H, m);  
5.55 (1H, q); 4.55 (2H, d); 3.35 (3H, s). MS TOF 402  
15 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 11.87 min.

##### Example 166.

R-2-{[N-(3-Amidino)benzoyl]amino}-2-phenylethyl-4-  
20 dimethylaminobenzoate

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.20 (1H, s); 8.05 (1H, d); 7.90 (1H,  
d); 7.75 (2H, d); 7.65 (1H, t); 7.45 (2H, d); 7.35 (3H,  
m); 6.70 (2H, d); 5.50 (1H, m); 4.55 (2H, m); 1.95  
25 (6H, s). MS TOF 431 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient  
3, water/acetonitrile/TFA) rt 11.11 min.

##### Example 167.

R-2-{[N-(3-Amidino)benzoyl]amino}-2-phenylethyl-4-  
30 aminosulphonylbenzoate

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.15 (1H, s); 8.05 (3H, m); 7.90 (3H, m);  
7.65 (1H, t); 7.55 (2H, d); 7.35 (3H, m); 5.50 (1H, m);  
4.65 (2H, d). MS TOF 467 (M+1<sup>+</sup>). Hplc (Jupiter5 C18,  
35 Gradient 3, water/acetonitrile/TFA) rt 9.80 min.

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**Example 168.****R-2-{[N-(3-Amidino)benzoyl]amino}-2-phenylethyl benzoate**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.05 (1H, s); 8.00 (1H, d); 7.80 (2H, m);  
5 7.70 (1H, d); 7.55 -7.00 (9H, m); 5.45 (1H, m); 4.55  
(2H, m). MS TOF 387 (M+1<sup>+</sup>). Hplc (Jupiter5 C18,  
Gradient 3, water/acetonitrile/TFA) rt 10.85 min.

**Example 169.**

10 **R-2-{[N-(3-Amidino)benzoyl]amino}-2-phenylethyl-4-  
acetoxybenzoate**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.00 (1H, s); 7.95 (1H, d); 7.85 (2H, d);  
7.75 (1H, d); 7.55 (1H, t); 7.35 (2H, d); 7.20 (3H, m);  
15 7.00 (2H, d); 5.35 (1H, m); 4.55 (2H, m); 2.10 (3H, s).  
MS TOF 446 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 10.54 min.

**Example 170.**

20 **R-2-{[N-(3-Amidino)benzoyl]amino}-2-phenylethyl-4-  
isopropylbenzoate**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.05 (1H, s); 8.00 (1H, d); 7.90 (1H, d);  
7.75 (2H, d); 7.55 (1H, t); 7.40 (2H, d); 7.25 (5H, m);  
25 5.45 (1H, m); 4.55 (2H, m); 2.85 (1H, m); 1.10 (6H, d). MS  
TOF 430 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 12.43 min.

**Example 171.**

30 **R-2-{[N-(3-Amidino)benzoyl]amino}-2-phenylethyl-4-  
hydroxybenzoate**

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.15 (1H, s); 8.05 (3H, m); 7.85 (1H, d);  
8.80 (2H, d); 7.65 (1H, t); 7.40 (5H, m); 6.80 (2H, d);  
35 5.50 (1H, m); 4.55 (2H, d). MS TOF 404 (M+1<sup>+</sup>). Hplc  
(Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt  
10.05 min.

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The following compounds were synthesised by Method 4 and elaborated as Example 89

Example 172.

5    2-Amino-5-amidino-benzoyl-D-phenylglycine benzylamide

<sup>1</sup>H nmr (DMSO) 8.30 (1H, s); 7.80 (1H, d); 7.75 (2H, d);  
7.45 (8H, m); 7.05 (1H, d); 5.95 (1H, s); 4.55 (2H, s).  
MS TOF 402 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1,  
10    water/acetonitrile/TFA) rt 10.55 min.

Example 173.

2-Amino-5-amidino-benzoyl-D-phenylglycine-2-  
methylbenzylamide

15    <sup>1</sup>H nmr (DMSO) 8.15 (1H, s); 7.70 (1H, d); 7.60 (2H, d);  
7.30 (7H, m); 6.90 (1H, d); 5.80 (1H, s); 4.30 (2H, s);  
2.25 (3H, s). MS TOF 416 (M+1<sup>+</sup>). Hplc (Jupiter5 C18,  
Gradient 1, water/acetonitrile/TFA) rt 10.07 min.

Example 174.

2-Amino-5-amidino-benzoyl-D-phenylglycine-3-  
methylbenzylamide

25    <sup>1</sup>H nmr (DMSO) 8.15 (1H, s); 7.60 (1H, d); 7.50 (2H, d);  
7.35 (4H, m); 7.15 (1H, m); 6.95 (2H, m); 6.80 (1H, d);  
5.70 (1H, s); 4.30 (2H, s); 2.25 (3H, s). MS TOF 416  
(M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1,  
water/acetonitrile/TFA) rt 10.94 min.

Example 175.

2-Amino-5-amidino-benzoyl-D-phenylglycine-R- $\alpha$ -  
methylbenzylamide

35    <sup>1</sup>H nmr (DMSO) 8.15 (1H, s); 7.60 (1H, d); 7.50 (2H, d);  
7.35 (5H, m); 7.15 (1H, m); 6.95 (2H, m); 6.80 (1H, d);  
5.70 (1H, s); 5.00 (1H, m); 1.45 (3H, d). MS TOF 416

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(M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/TFA) rt 10.58 min.

**Example 176.**

5    **2-Amino-5-amidino-benzoyl-D-phenylglycine-1-naphthylamide**

<sup>1</sup>H nmr (DMSO) 8.00 (1H, s); 7.85 (2H, m); 7.75 (1H, d);  
7.50 (1H, d); 7.40 (4H, m); 7.25 (5H, m); 6.70 (1H, d);  
10    5.60 (1H, s); 4.65 (2H, s). MS TOF 452 (M+1<sup>+</sup>). Hplc  
(Jupiter5 C18, Gradient 1, water/acetonitrile/TFA) rt  
11.17 min.

**Example 177.**

15    **2-Amino-5-amidino-benzoyl-D-phenylglycine-2-methylcyclohexylamide**

<sup>1</sup>H nmr (DMSO) 8.00 (1H, s); 7.50 (1H, d); 7.40 (2H, m);  
7.25 (4H, m); 6.70 (1H, d); 5.55 (1H, s); 3.20 (1H, m);  
20    1.7 -0.7 (9H, broad m) 0.70 (3H, d). MS TOF 408 (M+1<sup>+</sup>).  
Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/TFA)  
rt 10.95 min.

**Compounds synthesised by Method 12**

25

**Example 178.**

**R-2-{[N-(3-Amidino)benzoyl]amino}-2-phenylethyl-4-methoxybenzamide**

30    Typically Boc-(R)-1,2-diamino-1-phenylethane was prepared by the Method of O'Brien, P. et al. *J. Med. Chem.* 37 (1994) 12, 1810-1822. The free amino group was reacted with an acid chloride or an activated acid, all couplings (minimum 120 min.) were carried out in DMF.  
35    After an aqueous work up the deprotection of the Boc group was achieved with TFA. Other acid substituents were added as the HOBT or HOAt esters either by

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activation with HBTU/HATU, EDC or DIPCI with or without Boc protection of amino groups. The final products were purified by preparative reverse phase Hplc.

- 5 Boc-(R)-1,2 diamino-1-phenylethane (2g) was dissolved in DCM (80ml) and toluoylchloride (1.2ml.) and TEA (1.1ml.) added. The mixture was stirred at room temperature under argon overnight. The mixture was then washed with 2M sodium hydroxide solution, water, 5% hydrochloric acid and brine, dried over magnesium sulphate, filtered and  
10 evaporated. The product was recrystallised from hexane dissolved in DCM (50ml.) and treated with TFA for 2hrs. The solvent was evaporated and the solid triturated with diethylether. Coupling to m-benzamidine as Method 4.
- 15 <sup>1</sup>H nmr (DMSO) 8.25 (1H, s); 8.20 (1H,d); 7.95 (1H, d); 7.75 (3H,m); 7.35 (5H, m); 6.90 (2H,d); 5.35 (1H, m); 3.80 (3H,s); 3.70 (2H,m). MS TOF 417 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt  
20 10.31 min.

**Example 179.**

**R-2-{[N-(3-Amidino)benzoyl]amino}-2-phenylethyl-4-methylbenzamide**

- 25 <sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.05 (1H, s); 8.00 (1H,d); 7.85 (1H, d); 7.65 (1H, t); 7.55 (2H,d); 7.35 (5H, m); 7.20 (2H,d); 5.30 (1H, m); 3.75 (2H,m); 2.30 (3H,s); MS TOF 401 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 10.58 min.
- 30



## Compounds synthesised by Miscellaneous Methods

Example 180.

5     3-Amidino-benzoyl-DL-(4-methoxycarbonyl)phenylglycine-4-methylbenzylamine

10     D,L-4-Carboxyphenylglycine was prepared from 4-cyanobenzaldehyde by a method described by Clark, B. P. et al *Biorganic & Medicinal Chemistry Letters*, 1997, 7, 2777-2780.

15     D,L-4-Carboxyphenylglycine (600mg.) was suspended in methanol (30ml) at 0°C, saturated with hydrogen chloride gas and left at room temperature overnight. The reaction mixture was then evaporated to dryness under reduced pressure, resuspended in methanol and evaporated twice more and the solid triturated with ether, filtered and dried to give D,L-4-methoxycarbonylphenylglycine methyl ester HCl salt 620mg.

20     D,L-4-methoxycarbonylphenylglycine methyl ester HCl salt (600mg) was reacted with di-t-butyl dicarbonate in dioxane/ aqueous sodium bicarbonate to give after extraction with ethyl acetate and flash chromatography (SiO<sub>2</sub> hexane/ethyl acetate) - D,L-Boc-4-methoxycarbonylphenylglycine methyl ester 590mg. - <sup>1</sup>H nmr (CDCl<sub>3</sub>) 7.78 (2H, d); 7.20 (2H, d); 5.61 (1H, broad d); 5.13 (1H, broad d); 3.63 (3H, s); 3.44 (3H, s); 1.16 (9H, s).

30     D,L-Boc-4-methoxycarbonylphenylglycine methyl ester (400mg) was dissolved in THF 10ml and treated with a solution of lithium hydroxide monohydrate 64mg. in water 1ml and the mixture stirred at room temperature for 35     30min. The reaction mixture was acidified with 2M hydrochloric acid, extracted with ethyl acetate and the organic solution was washed twice with water and dried

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(MgSO<sub>4</sub>). Evaporation of the solvent and purification by flash chromatography gave D,L-Boc-4-methoxycarbonyl-phenylglycine 282mg. <sup>1</sup>H nmr (CDCl<sub>3</sub>) 8.0 (1H, broad d); 7.78 (2H, d); 7.20 (2H, d); 5.13 (1H, broad d); 3.80 (3H, s); 1.13 (9H, s).

D,L-Boc-4-methoxycarbonylphenylglycine was coupled to 4-methylphenyl-benzylamine using TBTU/DIPEA/DMF in the usual manner to give D,L-(4-methoxycarbonyl)phenylglycine-4-methylphenylbenzylamide. <sup>1</sup>H nmr (CDCl<sub>3</sub>) 7.93 (2H, d); 7.35 (2H, d); 7.02 (2H, d); 6.93 (2H, d); 5.78 (2H, m); 5.08 (1H, broad s); 4.30 (2H, m); 3.86 (3H, s); 2.22 (3H, s); 1.30 (9H, s).

D,L-(4-methoxycarbonyl)phenylglycine-4-methylphenylbenzyl amide was deprotected using TFA/DCM and coupled to m-benzamidine carboxylic acid TFA salt as Method 4

<sup>1</sup>H nmr (DMSO) 8.40 (1H, s); 8.30 (1H, d); 8.10 (3H, m); 7.80 (3H, m); 7.20 (4H, s); 6.00 (1H, s); 4.35 (2H, s); 4.00 (3H, s); 2.40 (3H, s); MS TOF 459 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 1, water/acetonitrile/TFA) rt 10.88 min.

25

**Example 181.**

**3-(Trimethylacetyloxomethylcarbonylamidino)-benzoyl-D-phenylglycine-4-(4-chlorophenyl)piperazinamide**

Trimethylacetyloxymethyl carbonochloridate was prepared by the Method described by Folkmann and Lund, *Synthesis*, 1990, 1159., and reacted with 3-amidino-benzoyl-D-phenylglycine-4-(4'-chlorophenyl)piperazinamide (Example 116) in acetonitrile/aqueous sodium bicarbonate to give, after flash chromatography (SiO<sub>2</sub> - DCM/ethylacetate) trimethylacetyloxymethylcarbonyl-3-amidino-benzoyl-D-phenylglycine-4-(4'-chlorophenyl)piperazinamide.

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<sup>1</sup>H nmr (DMSO) 8.35 (1H, s); 8.10 (1H, d); 8.00 (1H, d);  
7.60 (1H, t); 7.55 (2H, m); 7.40 (3H, m); 7.20 (2H, d);  
6.80 (2H, d); 6.05 (1H, s); 5.75 (2H, s); 3.75 (1H, m);  
3.65 (2H, m); 3.45 (3H, m); 3.30 (1H, m); 2.85 (1H, m) 1.20  
5 (9H, s). MS TOF no (M+1<sup>+</sup>). Hplc (Jupiter5 C18,  
Gradient 3, water/acetonitrile/TFA) rt 13.30 min.

**Example 182.**

10 **R-3-Amidino-{2-(4-benzylpiperidin-1-yl)}-1-phenylethyl  
benzamide**

Boc-D-Phenylglycine-OH was coupled to 4-benzylpiperidine  
as Method 4 and the Boc group removed with TFA. The  
product was dissolved in dry THF and added dropwise to  
15 LiAlH<sub>4</sub> (1.2eq) suspended in dry THF (15ml) under  
nitrogen and refluxed under nitrogen for 20 hours. On  
cooling the reaction was carefully diluted with water,  
acidified to pH 1 (HCl), and washed with EtOAc. The  
aqueous solution was basified to pH 10 (solid Na<sub>2</sub>CO<sub>3</sub>),  
20 then extracted with EtOAc to obtain product which was  
further elaborated as Method 4.

<sup>1</sup>H nmr (CD<sub>3</sub>CN) 8.25 (1H, s); 8.15 (1H, d); 7.95 (1H, d);  
7.70 (1H, t) 7.55 - 7.10 (10H, m); 5.60 (1H, m); 3.70  
25 (2H, m); 3.30 (2H, m); 2.95 (2H, m); 2.55 (2H, m); 1.80  
(3H, m); 1.50 (2H, m). MS TOF 441 (M+1<sup>+</sup>). Hplc (Jupiter5  
C18, Gradient 1, water/acetonitrile/TFA) rt 13.34 min.

**Example 183.**

30 **3-(Benzyloxycarbonylamidino)-benzoyl-D-phenylglycine-4-  
(4-chlorophenyl)piperazinamide**

Example 116 was dissolved in DCM and DIPEA (2.2eq),  
then benzyl chloroformate (1.1eq) was added and stirred  
35 at room temperature for 45 mins. The reaction mixture was  
washed with water then chromatographed on silica gel 60  
eluting with 65% EtOAc/Hexane to give the pure product.

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<sup>1</sup>H nmr (CD<sub>3</sub>OD) 8.25 (1H, s); 8.05 (1H, d); 7.95 (1H, d);  
7.60 (1H, t); 7.45 (5H, m); 7.20 (2H, d); 6.80 (2H, d);  
6.10 (1H, s); 5.20 (2H, s); 3.80 (1H, m); 3.60 (3H, m);  
3.20 (2H, m); 2.95 (1H, m); 2.50 (1H, m). MS TOF 611  
5 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3,  
water/acetonitrile/TFA) rt 13.15 min.

**Example 184.**

3-(tert-Butyloxycarbonylamidino)-benzoyl-D-  
10 phenylglycine-4-(4-chlorophenyl)piperazinamide

Example 116 was dissolved in dioxane and NaHCO<sub>3</sub>  
(2.2eq.), di tertbutyl dicarbonate (1.1eq) was added and  
stirred at room temperature for 1 week. The reaction  
15 mixture was extracted with ethyl acetate and evaporated  
to dryness. The product was chromatographed on silica  
gel 60 eluting with 75% EtOAc/Hexane to give the pure  
product.

20 <sup>1</sup>H nmr (CD<sub>3</sub>OD) 8.25 (1H, s); 7.95 (2H, m); 7.55 (1H, t);  
7.45 (5H, m); 7.15 (2H, d); 6.80 (2H, d); 6.10 (1H, s);  
3.85 (3H, m); 3.60 (1H, m); 3.10 (2H, m); 2.90 (1H, m); 2.50  
(1H, m); 1.45 (9H, s). MS TOF 577 (M+1<sup>+</sup>). Hplc (Jupiter5  
C18, Gradient 1, water/acetonitrile/TFA) rt 12.72 min.

25

**Example 185.**

3-(Acetylamidino)-benzoyl-D-phenylglycine-4-(4-  
chlorophenyl)piperazinamide

30 Example 116 was dissolved in dichloromethane and DIPEA  
(2.2eq) added. Acetyl chloride (1.1eq) was added and  
stirred at room temp for 45 mins. The reaction mixture  
was then washed with water and chromatographed on  
silica gel 60 eluting with EtOAc. The product was  
35 further purified by preparative hplc.

<sup>1</sup>H nmr (CD<sub>3</sub>OD) 8.25 (1H, s); 8.05 (2H, m); 7.65 (1H, t);

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7.45 (5H, m); 7.25 (2H, d); 6.85 (2H, d); ; 6.15 (1H, s);  
3.65 (3H, m); 3.60 (1H, m); 3.20 (2H, m); 3.00 (1H, m); 2.55  
(1H, m); 2.40 (3H, s). MS TOF 519 (M+1<sup>+</sup>). Hplc (Jupiter5  
C18, Gradient 3, water/acetonitrile/TFA) rt 14.53 min.

5

**Example 186.****(R)-O-(3-Amidinobenzyl)-N-(4-methylbenzyl)mandelamide****(R)-O-(3-Cyanobenzyl) mandelic acid**

10 (R)-Mandelic acid 91g, 6.57mmol.) was dissolved in THF  
(15ml.) and treated portionwise with sodium hydride  
(60%w/w, 0.798g, 19.7mmol.). The white suspension was  
heated at reflux and treated dropwise with 3-  
15 cyanobenzylbromide (1.93g, 9.86mmol.) in THF (20ml.)  
over 5mins. The mixture was refluxed for 2 days. The  
reaction mixture was cooled and treated with water  
(100ml.). The aqueous phase was washed with diethyl  
ether then acidified to pH 1 with 10% HCl solution. The  
20 product was extracted with ethyl acetate. The extracts  
were washed with brine and dried over magnesium  
sulphate, filtered and evaporated to give the product  
(1.5g) as a gum.

25 <sup>1</sup>H nmr (CD<sub>3</sub>OD) 7.70 (3H, m); 7.40 (6H, m); 5.00 (1H, s);  
4.65 (2H, m).

**(R)-O-(3-Cyanobenzyl)-N-(4-methylbenzyl)mandelamide**

30 HOAt (0.27g, 1.96mmol.) and DBU (0.3g, 1.96mmol.) was  
dissolved in DMF (8ml.) and cooled in ice. EDC (0.38g,  
1.96mmol.) was added in 3 portions and stirred for  
10mins.. To this mixture was added (R)-O-(3-Cyanobenzyl)  
mandelic acid (0.5g, 1.87mmol.) in DMF (2ml.) and the  
mixture allowed to warm to room temperature for 5 mins.  
35 4-Methylbenzylamine (0.23g, 1.96mmol.) was then added in  
one portion. The mixture was stirred for 1 hr. The  
reaction was quenched with water (100ml.) and basified

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with 2M sodium hydroxide solution. The product was extracted with ethylacetate. The combined extracts were washed with dilute (5%) lithium bromide solution, water and brine, dried over magnesium sulphate, filtered and evaporated to give a gum (0.58g). The crude product was chromatographed on silica gel eluting with 35% ethylacetate in hexane.

<sup>1</sup>H nmr (CDCl<sub>3</sub>) 7.50 (2H,m); 7.30 (6H,m); 7.05 (4H,s); 6.90 (1H,m); 4.80 (1H,s); 4.55 (2H,m) 4.35 (2H,s); 2.25 (3H,s).

**(R)-O-(3-Amidinobenzyl)-N-(4-methylbenzyl)mandelamide**

(R)-O-(3-Cyanobenzyl)-N-(4-methylbenzyl)mandelamide (0.16g) was dissolved in ethanol (10ml) and treated with hydrogen chloride gas for 30 mins. The mixture was then stirred overnight at room temperature, evaporated to dryness and dissolved again in ethanol (10ml.). The mixture was treated with ammonia gas for 5 mins. and allowed to stand overnight. The mixture was evaporated and the product purified by preparative hplc.

<sup>1</sup>H nmr (CDCl<sub>3</sub>) 7.65 (1H,s); 7.50 (1H,d ); 7.30 (7H,m); 7.00 (4H,s); 4.80 (1H,s); 4.55 (2H,m) 4.35 (2H,s); 2.25 (3H,s). MS TOF 387 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 11.24 min.

**Example 187.**

**2-(3-Amidinobenzoyl)-1-(3-phenylpropanoyl)-1-phenylhydrazine.**

**1-(3-phenylpropanoyl)-1-phenylhydrazine**

2-tert Butoxycarbonyl-1-phenylhydrazine (1.3g) was dissolved in pyridine (6ml.) and treated with 3-phenylpropanoyl chloride (0.75ml.). The mixture was stirred overnight at room temperature under argon. The pyridine was then removed by evaporation and the residue

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taken up in ethyl acetate and washed with 10% hydrochloric acid, saturated sodium carbonate solution and brine. The ethyl acetate fraction was dried over magnesium sulphate, filtered and evaporated. The crude product was chromatographed on silica eluting 5-25% ethyl acetate in hexane. The purified product was dissolved in DCM and treated with TFA before coupling to m-benzamidine by Method 4.

<sup>1</sup>H nmr (CD<sub>3</sub>OD) 8.20 (1H, s); 8.05 (1H, d); 7.90 (1H, d); 7.65 (1H, t); 7.35 (5H, m); 7.10 (5H, m); 6.15 (1H, s); 2.60 (4H, m). MS TOF 388 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 10.75 min.

**Example 188.**

3-{[R]-N-(3-Amidinobenzamido)-1-phenyl}methyl-5-benzyl-1,2,4-triazole.

EDC (1.6g) was dissolved in DMF (20ml.) and treated with HOAt (1.16g) in DMF (10ml.) and stirred for 10 mins. DIPEA (1.46ml.) was then added and the stirring continued for a further 15 mins. Boc-D-phenylglycine (2g) in DMF (10ml.) was then added dropwise and stirring continued for a further 25mins. 1M Hydrazine in THF (84ml.) was then added and the mixture stirred over night. The solvents were then evaporated and the residue treated with water (100ml.), basified with 2M sodium hydroxide solution and extracted with ethyl acetate. The combined extracts were washed with water and brine, dried with magnesium sulphate, filtered and evaporated to give a yellow solid (1.38g).

<sup>1</sup>H nmr (CDCl<sub>3</sub>) 7.20 (5H, s); 5.55 (1H, s); 1.25 (9H, s).

The above product (0.3g, 1.13mmol.) was dissolved in ethanol (6ml.) and added dropwise to a solution of benzyl ethyl imidate hydrochloride in ethanol (3ml.) at

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room temperature. The mixture was stirred for 30 mins. The solvent was then evaporated and the residue treated with dilute sodium bicarbonate solution and extracted with ethylacetate. The combined extracts were washed  
5 with brine, dried over magnesium sulphate, filtered and evaporated to give a yellow gum. (0.43g).

<sup>1</sup>H nmr (CDCl<sub>3</sub>) 7.30 (10H,m); 5.80 (1H,s); 3.50 (2H,m);  
1.45 (9H,s).

10

The above crude product (0.43g, 1.12 mmol.) was dissolved in xylene (60ml.) with para toluenesulphonic acid (cat.) and heated to 160°C for 1 day. The xylene was removed under vacuo and the triazole (0.35g) used  
15 without further purification.

<sup>1</sup>H nmr (CDCl<sub>3</sub>) 7.20 (10H,m); 5.70 (1H,s); 4.00 (2H,m);  
1.30 (9H,s).

20

The above product was treated with TFA and coupled to m-benzamidine by Method 4

<sup>1</sup>H nmr (CD<sub>3</sub>OD) 8.20 (1H,s); 8.05 (1H,d); 7.80 (1H,d);  
7.60 (1H,t); 7.20 (10H,m); 6.50 (1H,s); 4.10 (2H,s).

25

MS TOF 411 (M+1<sup>+</sup>). Hplc (Jupiter5 C18, Gradient 3, water/acetonitrile/TFA) rt 14.38 min.

#### References:

30

(1) (a) Bucherer, H. T.; Steiner W. *J. Prakt. Chem* 1934, 140, 291-316.

(b) Greene T. W.; Wuts P. G. M. *Protective Groups in Organic Synthesis*, (John Wiley and Sons, Inc. 1991) 318-319 and references therein.

35

(c) Bolin D. R.; Sytwu I.; Humiec F.; Meienhofer J. *Int. J. Peptide Protein Res.* 1989, 33, 353-359.



- (2) Shearer, B. G., Oplinger, J. A., Lee, S. *Tet. Letts.* 38(2), 179-182 (1997)
- (3) Chandrakumar, N.S. *Synthetic Comms.*, 26(14), 2613-2616 (1996)
- 5 (4) Bundle et al *J. Chem, Soc. Perkin Trans I* 1985 2247)

### Assay protocols

#### Enzyme Inhibition assays:

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Enzyme assays were carried out at room temperature in 0.1M phosphate buffer, pH7.4 according to the method of Tapparelli et al (*J. Biol. Chem.* 1993, 268, 4734-4741). Purified human factor Xa, trypsin, thrombin and plasmin were purchased from Alexis Corporation, Nottingham, UK. Urokinase was purchased from Calbiochem, Nottingham, UK. Chromogenic substrates for these enzymes; pefachrome-FXA, pefachrome-TRY, pefachrome-TH, pefachrome-PL and pefachrome-UK were purchased from Pentapharm AG, Basel, Switzerland. Product (*p*-nitroaniline) was quantified by adsorption at 405nm in 96 well microplates using a Dynatech MR5000 reader (Dynex Ltd, Billingshurst, UK).  $K_m$  and  $K_i$  were calculated using SAS PROC NLIN (SAS Institute, Cary, NC, USA, Release 6.11)  $K_m$  values were determined as 100.9  $\mu M$  for factor Xa/pefachrome-FXA and 81.6  $\mu M$  for trypsin/pefachrome-TRY. Inhibitor stock solutions were prepared at 40mM in Me2SO and tested at 500  $\mu M$ , 50  $\mu M$  and 5  $\mu M$ . Accuracy of  $K_i$  measurements was confirmed by comparison with  $K_i$  values of known inhibitors of factor Xa and trypsin.

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In agreement with published data, benzamidine inhibited factor Xa, trypsin, thrombin, plasmin and urokinase with  $K_i$  values of 155  $\mu M$ , 21  $\mu M$ , 330nM, 200nM and 100nM respectively. NAPAP inhibited thrombin with a  $K_i$  value of 3nM. Compounds of the invention were found to have activity in these assays.

35

### Antithrombotic Activity

The test material (Factor Xa inhibitor) was administered either intravenously, intraperitoneally or orally to a group of rats for experiment. A second group received vehicle (saline) only as a control, and a third group of animals received a standard antithrombotic (subcutaneous lmw heparin) as a positive control.

To perform the experiment male Sprague-Dawley rats (250-400g in weight) were anaesthetised by the inhalation of isoflurane with the addition of oxygen and nitrous oxide. The left or right femoral vein was carefully exposed and isolated from the femoral artery and saphenous nerve. Following removal of connective tissue a cannula containing physiological saline was inserted into the femoral vein.

A segment of each of the left and right jugular vein was exposed and separated from the surrounding connective tissue. Each segment consisted of the section of vein between the exit point from the thorax to the vessel's first major junction.

At the desired interval following the administration of the test material or vehicle a bolus injection of 'deactivated' human serum ( $1.32 \text{ ml.kg}^{-1}$ ) was administered over less than 30 seconds, via the femoral vein cannula. Two minutes following the thrombus challenge both the jugular vein segments were ligatured at both ends and left *in situ* for thrombi to form.

After 10 min both jugular segments were carefully excised, and placed in a petri dish containing 0.9% saline. A blood sample (1.8 ml blood + 0.2 ml 3.8% sodium citrate) was obtained by cardiac puncture and the animal sacrificed by an overdose of Expiral (sodium

pentobarbitone) administered intravenously via the femoral vein cannula or by cardiac puncture. The 2 segments of jugular vein were carefully dissected longitudinally along one surface to expose the lumen and dispel the vessel contents into the saline. The tissues were examined for the presence of any developed thrombi and scored accordingly.

Thrombus score:

10

- 0 = No thrombus
- 1 = One or several small thrombi
- 2 = Several larger thrombi
- 3 = Large thrombus occluding the vessel

15

Compounds of the invention were found to have significant antithrombotic activity in these assays.

Partial Thromboplastin Time Test Protocol

20

Venous blood was collected into 3.2% (0.109m) trisodium citrate vacutainer tubes at 1 volume of anticoagulant to nine volumes of blood.

25

The blood cells were separated by centrifugation at 700g for ten minutes to yield plasma, which was frozen at 70°C until required.

30

To perform the test, 100µl of plasma was pipetted into in a glass test tube, 1µl of test compound in DMSO was added, and allowed to warm to 37° over two minutes.

35

100µl of warm (37°) Manchester (tissue thromboplasin) reagent (Helena Biosciences, UK) was added, allowed to equilibrate for sixty seconds.

100µl of warm (37°) 25mM calcium chloride solution was added to initiate clotting.

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The test tube was tilted three times through a 90° angle every five seconds to mix the reagents and the time to clot formation recorded.

- 5 Data from a series of observations and test compound concentrations are analysed by a SAS statistical analysis program and a CT2 (Concentration required to double clotting time) for each compound is generated.
- 10 Compounds of the invention were found to significantly elongate the partial thromboplastin time.

**Kaolin Activated Partial Thromboplastin Time Test Protocol**

- 15 Venous blood was collected into 3.2% (0.109m) trisodium citrate vacutainer tubes at 1 volume of anticoagulant to nine volumes of blood.
- 20 The blood cells were separated by centrifugation at 700g for ten minutes to yield plasma, which was frozen at 70°C until required.
- 25 To perform the test, 100µl of plasma was pipetted into in a glass test tube, 1µl of test compound in DMSO was added, and allowed to warm to 37° over two minutes.
- 30 100µl of APTT reagent resuspended in Owren's buffer (Thrombosis Reference Centre University of Manchester, Withington Hospital, Manchester) was added, mixed thoroughly, and 100µl of prewarmed kaolin suspension in Owren's buffer added.
- 35 This mixture was incubated for ten minutes at 37°, with regular gentle mixing by tilting through a 90° angle, three times sequentially at one minute intervals to maintain the kaolin in suspension.

After ten minutes 100 $\mu$ l of 25mM calcium chloride solution was added, the suspension redispersed by mixing as above and the time to clot formation recorded.

5

Data from a series of observations and test compound concentrations are analysed by a SAS statistical analysis program and a CT2 (Concentration required to double clotting time) for each compound is generated.

10

Table 1 Biological data for selected examples

15	EX No	FXa	thrombin	trypsin	APTT	PT
		pKi	pKi	pKi	CT2 uM	CT2 uM
	84	7.98	5.04	5.61	5.8	1.9
	89	7.85	5.99	6.61	NT	NT
	63	7.80	5.96	6.54	NT	3.7
	87	7.62	5.10	5.89	14	4.2
20	33	7.50	6.14	6.39	NT	7.1
	32	7.39	6.09	5.65	7.4	5.6
	65	7.19	5.93	5.79	9.7	13
	36	7.12	6.24	6.16	9.4	9
	64	7.06	5.72	5.91	7.6	8.3
25	68	7.01	5.32	5.59	NT	17
	66	6.94	6.66	6.55	4.5	4.6
	31	6.80	6.98	5.56	3.7	3.2
	37	6.79	4.77	5.84	36	15
	80	6.57	5.16	5.73	NT	13
30	12	6.39	5.27	6.10	6.5	3.4

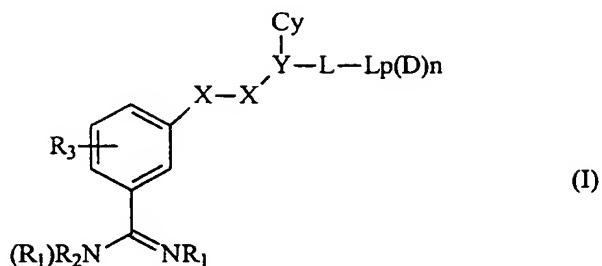
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## Claims

1. A serine protease inhibitor compound of formula I

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(where  $R_1$  and  $R_2$  each independently is hydrogen or hydroxyl, alkoxy, alkyl, aminoalkyl, hydroxyalkyl alkoxyalkyl, alkoxycarbonyl, acyloxymethoxycarbonyl or alkylamino optionally substituted by hydroxy, alkylamino, alkoxy, oxo, aryl or cycloalkyl;

each  $R_3$  independently is  $R_1$ ,  $R_2$ , amino, halo, cyano, nitro, thiol, alkylthio, alkylsulphonyl, alkylsulphenyl, alkylsulphonamido, alkylaminosulphonyl, haloalkoxy and haloalkyl;

each X independently is a C, N, O or S atom or a CO,  $CR_1$ ,  $C(R_1)_2$  or  $NR_1$  group, at least one X being C, CO,  $CR_1$  or  $C(R_1)_2$ , with the proviso that if the benzamidine group is unsubstituted and the X-X group is  $-CH_2C(R_1)_2-$  then  $R_1$  is hydrogen or is attached to the alkylene carbon atom by a heteroatom;

L is an organic linker group containing 1 to 5 backbone atoms selected from C, N, O and S, or a branched alkyl or cyclic group;

Y is a nitrogen atom or a  $CR_1$  group or Y and L taken together form a cyclic group;

Cy is a saturated or unsaturated, mono or poly cyclic, homo or heterocyclic group optionally substituted by groups  $R_3$  or phenyl optionally substituted by  $R_3$ ;

Lp is a lipophilic organic group, selected from an

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alkyl, heterocyclic, alkenyl, alkaryl, cycloalkyl, polycycloalkyl, cycloalkenyl, aryl, aralkyl or haloalkyl group or a combination of two or more such groups optionally substituted by one or more of oxa, oxo, aza, thio, halo, amino, hydroxy or  $R_3$  groups;

D is a hydrogen bond donor group; and n is 0, 1 or 2)

or a physiologically tolerable salt thereof.

2. A compound as claimed in claim 1 wherein  $R_1$  represents hydrogen.

3. A compound as claimed in either one of claims 1 or 2 wherein  $R_2$  is hydrogen, alkoxycarbonyl or hydroxy.

4. A compound as claimed in any one of claims 1 to 3 wherein  $R_3$  is hydrogen, amino, aminoalkyl, alkylamino, hydroxy, hydroxyalkyl, thiol, alkylthio or alkoxy.

5. A compound as claimed in claim 4 wherein  $R_3$  is para to the amidino group.

6. A compound as claimed in claim 5 wherein  $R_3$  is amino.

7. A compounds as claimed in any one of claims 1 to 6 wherein the linker X-X is -CH=CH-, -CONH-, -CONR<sub>1</sub>-, -NH-CO-, -NH-CH<sub>2</sub>-, -CH<sub>2</sub>-NH-, -CH<sub>2</sub>O-, -OCH<sub>2</sub>-, -COO-, -OC=O- or -CH<sub>2</sub>CH<sub>2</sub>-.

8. A compound as claimed in claim 7 wherein the X moiety nearest Y is NH.

9. A compound as claimed in claim 7 wherein the X moiety nearest the phenyl ring is CH<sub>2</sub> or CO.

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10. A compound as claimed in claim 7 wherein X-X is  
-CONH-.
11. A compound as claimed in any one of claims 1 to 10  
5 wherein Y is CH.
12. A compound as claimed in any one of claims 1 to 11  
wherein Cy represents aryl or heteroaryl optionally  
substituted by  $R_3$ .
- 10 13. A compound as claimed in claim 12 wherein  $R_3$   
represents phenyl optionally substituted by  $R_3$  or naphthyl  
optionally substituted by  $R_3$ .
- 15 14. A compound as claimed in claim 13 wherein Cy  
represents phenyl.
15. A compounds as claimed in any one of claims 1 to 14  
wherein the linker L represents CO,  $\text{CH}_2\text{NH}$ ,  $\text{CONR}_1(\text{CH}_2)_m$ ,  
20  $(\text{CH}_2)_m\text{N}(\text{R}_1)\text{CO}(\text{CH}_2)_m$ ,  $(\text{CH}_2)_{m+2}$ ,  $(\text{CH}_2)_m\text{CO}(\text{CH}_2)_m$ ,  $(\text{CH}_2)_m\text{OC}=\text{O}$ ,  
 $(\text{CH}_2)_m\text{O}$  or  $\text{CH}=\text{CH}(\text{CH}_2)_m$  (where each m is independently 0 or  
1).
16. A compound as claimed in claim 15 wherein the  
25 linker L represents  $\text{CONR}_1(\text{CH}_2)_m$ , CO or  $\text{CH}_2\text{NR}_1\text{CO}$  where m is  
0 or 1.
17. A compound as claimed in any one of claims 1 to 16  
wherein said lipophilic group comprises cycloalkyl,  
30 azacycloalkyl, diazacycloalkyl, phenyl, naphthyl,  
adamantyl, decaliny, tetrahydrodecaliny, bicycloalkyl,  
mono- or diazabicycloalkyl, mono- or bicyclo  
heteroaromatic or a linear or branched alkyl, alkylene,  
alkenyl or alkenylene group all optionally substituted  
35 by one or more groups  $R_3$ , or a combination of at least  
two such groups linked by a spiro linkage or a single or  
double bond or by C=O, O, S, SO, SO<sub>2</sub>,  $\text{CONR}_1$ ,  $\text{NR}_1\text{-CO-}$ ,  $\text{NR}_1$



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linkage.

18. A compound as claimed in claim 17 wherein the lipophilic groups is a methyl-cyclohexyl, methylcyclohexylmethyl, methylphenylmethyl, phenylethyl, benzylpiperidinyl, benzoylpiperidinyl, bispiperidinyl or phenylpiperazinyl.

19. A compound as claimed in any one of claims 1 to 18 wherein the hydrogen bond donor group is attached to the lipophilic group.

20. A compound as claimed in claim 19 wherein nitrogen or oxygen is the hydrogen bond donor atom.

21. A compound as claimed in claim 20 wherein the hydrogen bond donor is formed from a hydroxyl group, a primary, secondary or tertiary amine, or a primary or secondary imine group as part of an amidine or guanidine or a saturated or unsaturated heterocyclic group containing 5 to 7 ring atoms and having a ring nitrogen.

22. A compound as claimed in any one of claims 1 to 21 wherein Y is carbon and preferably has the conformation that would result from construction from a D- $\alpha$ -aminoacid  $\text{NH}_2\text{-CR}_1(\text{Cy})\text{-COOH}$ .

23. A compound as claimed in any one of claims 1 to 22 wherein the serine protease to be inhibited is trypsin, thrombin, urokinase or Factor Xa.

24. A compound as claimed in claim 1 being 3-Amidino-benzoyl-D-phenylglycine-4-benzoyl piperidinamide.

25. A pharmaceutical composition comprising a serine protease inhibitor as claimed in any one of claims 1 to 24 together with at least one pharmaceutically

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acceptable carrier or excipient.

26. A serine protease inhibitor as claimed in any one  
of claims 1 to 24 for use in combatting a condition  
5 responsive to said inhibitor.

27. Use of a serine protease inhibitor as claimed in  
any one of claims 1 to 24 for the manufacture of a  
medicament for use in a method of treatment of the human  
10 or non-human animal body to combat a condition  
responsive to said inhibitor.

28. A compound as claimed in claim 27 wherein said  
condition is a thrombotic disease, asthma, emphysema,  
15 cirrhosis, arthritis, carcinoma, melanoma, restenosis,  
atheroma, trauma, shock and reperfusion injury.

29. A method of treatment of the human or non-human  
animal body to combat a condition responsive to a serine  
20 protease inhibitor, said method comprising administering  
to said body an effective amount of a serine protease  
inhibitor as claimed in any one of claims 1 to 24.

# INTERNATIONAL SEARCH REPORT

In. .onal Application No  
PCT/GB 98/02605

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC 6    C07K5/065    C07K5/087    C07K5/107    A61K38/05    A61K38/06 A61K38/07    C07C257/18    C07D209/20    C07D317/60    C07D333/24 C07D207/14    C07D211/26    C07D207/16    C07D233/54    C07D211/30					
According to International Patent Classification (IPC) or to both national classification and IPC					
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC 6    C07K    A61K    C07C    C07D					
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched					
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)					
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>					
Category *	Citation of document, with indication, where appropriate, of the relevant passages				Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 103, no. 25, 23 December 1985 Columbus, Ohio, US; abstract no. 209699z, XP002083330 see abstract & R. FERRONI ET AL.: FARMACO, ED. SCI., vol. 40, no. 10, 1985, pages 717-729, ---				1-28
Y	CHEMICAL ABSTRACTS, vol. 108, no. 7, 15 February 1988 Columbus, Ohio, US; abstract no. 48724q, XP002083331 see abstract & R. FERRONI ET AL.: FARMACO, ED. SCI., vol. 42, no. 10, 1987, pages 709-715, --- <div style="text-align: center;">-/-</div>				1-28
<div style="display: flex; justify-content: space-between;"> <span><input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.</span> <span><input checked="" type="checkbox"/> Patent family members are listed in annex.</span> </div>					
* Special categories of cited documents : <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>					
Date of the actual completion of the international search  <div style="text-align: center;">6 November 1998</div>			Date of mailing of the international search report  <div style="text-align: center;">24/11/1998</div>		
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016			Authorized officer  <div style="text-align: center;">Frelon, D</div>		